1992 World Congress on Cell and Tissue Culture

GENETIC APPLICATIONS OF TISSUE CULTURE

Host Sponsor:
Tissue Culture Association

Participating Organizations:

European Tissue Culture Society
American Type Culture Collection
European Society for Animal Cell Technology
UNESCO Biotechnology Advisory Committee
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Japanese Society of Plant Tissue Culture
Keystone Center
Korean Society of Plant Tissue Culture
Japanese Tissue Culture Association
U.S.A., Canadian, and Japanese Sections
of the International Association
for Plant Tissue Culture
International Service for the Acquisition
of Agri-Biotech Applications

20-25 June 1992
Hyatt Regency Crystal City Hotel, Arlington, VA (Washington, D.C.)

ORGANIZING COMMITTEE

David W. Altman (*Chair*)
Olivia Pereira-Smith (*Vice Chair*)
Daniel C. W. Brown
Ann Fallon
Eugene L. Elmore
Elliot Levine
Sandro Eridani
John Harbell
Robert Lawrence
Gary Pace

SATURDAY: 20 June **Potomac II**
16:30-19:00

SCIENTIFIC COMMITTEE MEETING

17:00-19:00 HISTORY SOCIETY PROGRAM **Regency B**

17:00-19:00 WHITE MEMORIAL AWARD COMMITTEE MEETING **Potomac I**

SATURDAY: 20 June **Regency E-F**
20:00-22:00

OPENING RECEPTION
All Registrants are Invited to Attend

Numbers preceeding names refer to abstracts.
Capitalization identifies speaker.

43rd Annual Meeting of the
Tissue Culture Association

Key to Letters Preceeding Session Title

I	=	Invertebrate Cells	T	=	Cellular Toxicology
JS	=	Joint Session	V	=	Vertebrate Cells
P	=	Plant Cells	W	=	Workshop
PS	=	Plenary Session			

SUNDAY: 21 June **Regency E-F**

PLENARY SESSION

Gene Transfer
Convener: Erwin Wagner, Research Institute of Molecular Biology, Austria

8:00 Micro-Targeting Allows for Precise and Routine Acceleration of Foreign Genes into Shoot Meristems of Cereals.
PS-1 I. POTRYKUS, R. Bilang, V. Iglesias, A. Gisel, S. Zhang, D. Stein, N. Leduc, and C. Sautter.
8:30 Cytokine Stimulation and Cell Interactions Affect Retroviral Vector-Mediated Gene Transfer into Mouse and Human Hematopoietic Stem Cells.
PS-2 J. W. BELMONT, K. Moore, and F. Fletcher.
9:00 Electroporation as Compared to Conventional Gene Transfer Techniques in the Study of Gene Function.
PS-3 D. SPANDIDOS.

COFFEE BREAK
9:45-10:30 a.m.

★ **LAST CHANCE** ★
Monday 12:00 noon
Exchange Banquet Voucher for Wednesday 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!

SUNDAY: 21 June **Regency E**

CONTRIBUTED PAPERS

P Plant Molecular Biology
Conveners: Roberta H. Smith, Texas A&M University, USA and K. Oono, National Institute of Agrobiological Resource, Japan

10:30 Splicing Abnormality of a Chloroplast Gene Transcript in Albino Pollen Plants in Rice.
P-1001 K. OONO, I. Maruta, and S. Kikuchi.
10:45 Genetic Engineering of Osmolyte Biosynthesis Genes in Tobacco.
P-1002 R. K. JAIN, S. Jana, and G. Selvaraj.
11:00 Characterizations of Rice Cell Suspension Cultures of Mutants for Lysine and Protein Levels.
P-1003 G. W. SCHAEFFER.
11:15 Accumulation of Flavonoids is Essential for UV-Resistance in *Arabidopsis*.
P-1004 R. LOIS and B. Buchanan.
11:30 Differential Response of Resistant and Susceptible Wheat Calli Cultures to Russian Wheat Aphid Phytotoxin.
P-1005 M. M. RAFI and R. S. Zemetra.
11:45 Somatic Hybrids and Cybrids Between *Senecio fuchsii* Gmel. and *Senecio jacobaea* L.
P-1006 G. R. WANG and H. Binding.
12:00 Molecular and Quantitative Studies on Foreign Gene Expression.
P-1007 R. H. SMITH and E. C. Ulian.
12:15 Expression of the Sulfur-Rich Brazil Nut 2S Albumin in Transgenic *Vicia narbonensis* Plants.
P-1008 T. PICKARDT, I. Saalbach, F. Machemehl, G. Saalbach, O. Schieder, and K. Müntz.

SUNDAY: 21 June **Washington A-B**

CONTRIBUTED PAPERS

P Plant Micropropagation

Conveners: Mark Bridgen, University of Connecticut, USA and Nidia Guzman, Agricultural Services and Development, Costa Rica

10:30 Somatic Embryogenesis and Complete Plantlet Regeneration in Oil Palm from Inflorescence Explants.
P-1009 N. GUZMAN and S. Umaña.

10:45 Coffee Somatic Embryogenesis in Liquid Cultures.
P-1010 M. R. SÖNDAHL and C. Noriega.

11:00 Observation of Cultured Tissues of Plant by the Cell Processor with Laser.
P-1011 Y. TAHAMA.

11:15 Micropropagation of *Citrullus lanatus* in a Membrane-Based, Continual-Flow Liquid Bioreactor.
P-1012 J. W. ADELBERG, B. B. Rhodes, S. A. Hale, and R. E. Young.

11:30 Micropropagation of Flowering Dogwood (*Cornus florida* L.): Effect of Mineral Source and Hormone Level.
P-1013 V. DECLERCK and S. S. Korban.

11:45 In Vitro Nutritional Studies to Develop an Optimum Growing Medium for *Alstroemeria*.
P-1014 M. P. BRIDGEN, G. C. Elliott, and M. A. Smith.

12:00 Standardization of Growth Regulators (6-BA; NAA) to Improve Rapid In Vitro Liquid Propagation Medium for *Oxalis tuberosa* (Oca).
P-1015 J. PEÑAFIEL and R. Estrada.

12:15 Rapid Multiplication of "Thompson Seedless" Grapevine by In Vitro Culture of Shoot Apices and Axillary Buds.
P-1016 A. M. ALLAM, D. E. El-Rayes, and M. F. Mansour.

12:30 In Vitro Hardening of Red Raspberry Plantlets Through CO_2 Enrichment, Relative Humidity and Sucrose Reduction.
P-1017 R. DENG and D. Donnelly.

SUNDAY: 21 June **Regency C**

CONTRIBUTED PAPERS

T Toxicology I

Convener: Eugene Elmore, National Institute for the Advancement of In Vitro Sciences, USA

10:30 The Effects of Oxidant Stress on $[Ca^{2+}]_i$ Regulation in Mouse Epidermal JB6 Cells.
T-1001 P. T. JAIN, I. K. Berezesky, and B. F. Trump.

10:45 Development and Application of a Human Cell Line Expressing 5 cDNAs Encoding Xenobiotic-Metabolizing Enzymes.
T-1002 C. L. CRESPI, B. W. Penman, F. J. Gonzalez, H. V. Gelboin, and R. Langenbach.

11:00 Studies on the Efficacy of Chemopreventive Agents with the Bleomycin Assay.
T-1003 Z. TRIZNA and T. C. Hsu.

11:15 Cultured Mesangial Cells in the Study of Cadmium Nephrotoxicity.
T-1004 T. A. CHIN and D. M. Templeton.

11:30 In Vitro Differentiation of Hamster Pulmonary Neuroendocrine Cells.
T-1005 M. EMURA, A. Ochiai, M. Riebe-Imre, B. Panning, I. Paulini, U. Mohr, and D. L. Dungworth.

11:45 Effects of Exposure to Polychlorinated Biphenyls (PCBs) on Natural Cytotoxicity of Earthworm Coelomocytes.
T-1006 M. Suzuki, E. L. COOPER, G. S. Eyambe, A. J. Goven, L. C. Fitzpatrick, and B. J. Venables.

12:00 In Vitro Effect of Toxicants Derived from Polluted Sediments on Activities of Hemocytes from the Oyster, *Crassostrea virginica*.
T-1007 F.-L. E. CHU, A. Volety, and R. Hale.

12:15 A Colorimetric Assay for Assessment of Drug Sensitivities in African Trypanosomes.
T-1008 R. KAMINSKY and R. P. N. Wasike.

SUNDAY: 21 June **Regency A**

CONTRIBUTED PAPERS

V Cell Culture Technology

Convener: A. L. Spiering, Synthecon, Inc., USA

10:30 Urothelial Cell Growth In Vivo on Biodegradable Polymer Scaffolds.
V-1001 A. ATALA, J. P. Vacanti, A. B. Retik, and M. R. Freeman

10:45 Expressed-Differentiated Properties of Rabbit Kidney Proximal Tubule Cells in Primary Culture Grown in Hormonally-Defined Medium in Total Absence of Glucose and Insulin.
V-1002 F. COURJAULT, D. Leroy, and T. Toutain.

11:00 Extracellular Matrix Analogs as Growth Factor Delivery System.
V-1003 C. J. DOILLON and F. Roy.

11:15 NASA Bioreactor: A Unique In Vitro Fluid Culture System for Three-Dimensional Tissue Formation with Anchorage-Dependent Cells.
V-1004 S. R. GONDA, G. M. Marley, N. A. Schroedl, R. S. Tuan, R. K. Sinha, and M. Ingram.

11:30 In Vitro Analyses of Wound Healing Promotion by a Human Dermal Replacement.
V-1005 S. R. Slivka, L. LANDEEN, F. S. Zeigler, and R. L. Bartel.

11:45 Optimization of Antigen Production by HTLV-I Cell Lines Grown Under Serum and Serum-Free Supplemented Conditions.
V-1006 A. V. TREVINO, M. Pollman, C. Brooks, L. E. Mathes, and M. D. Lairmore.

12:00 A Novel In Vitro Model for Human Prostatic Cancer.
V-1007 J. T. Mendoza and A. L. SPIERING.

12:15 Human Mesothelioma Cell Line ZK 70, Showing Both, Endothelial and Epithelial Differentiation Within Protein-Free Medium.
V-1008 K.-H. BERGHÄUSER, B. Knoblauch, S. Menke, H. Burger, and A. Schulz.

SUNDAY: 21 June **Regency F**

CONTRIBUTED PAPERS

V Regulation of Gene Expression

Convener: Joseph Leighton, Peralta Cancer Research Institute, USA

10:30 Both the Substrate and the Cytoskeleton are Important in Directing Protein Secretion in MDCK Cells.
V-1009 L. M. PATRONE, J. R. Cook, and R. G. Van Buskirk.

10:45 Effects of NGF and Gamma-IFN on the Diverse Subpopulations in the Neuroblastoma Cell Line: SY5Y.
V-1010 J. RIDGE, D. Terle, and I. Levenbook.

11:00 Regulation of Mesangial Cell Growth by Matrix and Soluble Proteoglycans.
V-1011 D. M. TEMPLETON, M.-Y. Fan, and T. Miralem.

11:15 The Effects of Thermal Injury on Gene Expression and Ultrastructure of Rat Proximal Tubule Epithelium (PTE) In Vitro.
V-1012 N. Yamamoto, S. H. CHANG, I. K. Berezesky, and B. F. Trump.

11:30 The Substrate Can Affect the Net Synthesis and/or Retention of Laminin in NHEK Cells.
V-1013 J. R. COOK and R. G. Van Buskirk.

11:45 Histologic Patterns of Neoplastic Epithelia in Matrix and Gradient Culture.
V-1014 J. LEIGHTON.

12:00 The Human C/EBPα Promoter is Transactivated by a Related Protein, C/EBPβ.
V-1015 D. R. Wilson, L. R. Hendricks-Taylor, S. C. Juan, and G. J. DARLINGTON.

12:15 Optimization of TSH-Induced Gene Expression in Ovine Thyroid Gland Cells in Culture.
V-1016 P. R. KERKOF, C. Carter, D. Zamora, and J. Shook.

SUNDAY: 21 June **Potomac I-II**

SESSION-IN-DEPTH

I Unsolved Problems in Invertebrate Cell Culture
Conveners: Karl Maramorosch, Rutgers University, USA and Josef Řeháček, Slovak Academy of Sciences, Czechoslovakia

14:00 Introduction
14:10 Tick Cell Culture.
I-1 J. ŘEHÁČEK.
14:50 Leafhopper Cell Culture.
I-2 D. L. NUSS.
15:30 Low Cost Invertebrate Cell Culture Media.
I-3 J. L. VAUGHN.
16:10 From Shake Flask to Industrial Fermentor: Challenges of Large-Scale Insect Cell Culture.
I-4 B. W. BELISLE, E. L. Walls, C. Celeri, C. Knoch, V. Singer, and K. Tang.

SUNDAY: 21 June **Regency E**

SESSION-IN-DEPTH

P Crops for Tomorrow
Convener: Indra K. Vasil, University of Florida, USA

The latest developments in genetic engineering of plants for value added and other important agronomic traits will be discussed. The prospects for introduction of such crops into the market place before the end of the present decade will be assessed.

14:00 Crops for Tomorrow.
P-1 I. K. VASIL.
14:15 Transgenic Corn Controlling European Corn Borer.
P-2 M. FROMM.
15:00 Genetic Engineering for Fertility Control
P-3 J. LEEMANS.
15:45 Development of Crop Plants with Disease Resistance and Value Added Traits.
P-4 P. J. M. VAN DEN ELZEN, A. Hoekema, and B. J. C. Cornelissen.

SUNDAY: 21 June **Washington A-B**

SESSION-IN-DEPTH

Sponsored by the Industrial In Vitro Toxicology Group

T Industrial Application of In Vitro Toxicology
Convener: Jack M. Lipman, Hoffmann-La Roche, Inc., USA

14:00 Introduction
14:10 Integration of In Vitro Tests into Pharmaceutical Safety Assessment.
T-1 O. P. FLINT.
14:40 A Collaborative Approach to the Evaluation of Alternatives to the Eye Irration Test Using Chemical Intermediates.
T-2 D. M. GALEN.
15:10 Evaluating Heart, Liver, and Kidney Toxicity In Vitro with Precision-Cut Tissue Slices.
T-3 C. E. RUEGG, T. M. Greenwalt, and P. M. Silber..
15:40 Understanding Secondary Mechanisms of Carcinogenicity Using In Vitro Approaches.
T-4 J. M. LIPMAN.

SUNDAY: 21 June **Regency C**

SESSION-IN-DEPTH

T Strategies for Development and Evaluation of Antiviral Agents
Convener: Marie Chow, Massachusetts Institute of Technology, USA

14:00 Overview of Antiviral Strategies.
T-5 M. CHOW.
14:40 Molecular Mechanisms of Action of Ribavirin.
T-6 J. L. PATTISON.
15:20 Protein Acylation: Potential Targets for New Antiviral Therapies.
T-7 J. GORDON.
16:00 Use of Structure in the Design of Anti-Viral Drugs.
T-8 J. M. HOGLE.

SUNDAY: 21 June **Regency A**

SESSION-IN-DEPTH

Co-sponsored by the European Tissue Culture Society

V Multiple Drug Resistance in Cancer Cells
Convener: Martin Clynes, Dublin City University, Ireland

14:00 Expression of P-glycoprotein in Normal and Malignant Cells.
V-1 G. BRADLEY, S. Rajalakshmi, and V. Ling.
14:25 Molecular Analysis of the Multidrug Transporter.
V-2 M. M. GOTTESMAN.
14:50 Altered DNA Topoisomerase II in Multidrug Resistance.
V-3 W. T. BECK, M. K. Danks, J. S. Wolverton, M. Chen, B. Y. Bugg, and D. P. Suttle.
15:15 In Vitro and In Vivo Resistance in L1210 Murine Leukemia Cells to Novel Antitumor Compounds.
V-4 M. GRANDI, E. Pesenti, and C. Geroni.
15:40 Manipulating Cellular Drug Resistance with Genetic or Serological Reagents.
V-5 I. B. RONINSON, A. V. Gudkov, C. Zelnick, A. R. Kazarov, T. A. Holzmayer, and E. G. Mechetner.
16:05 Reversal of Adriamycin (Adr) Resistance by Lontidamine (LND) in a Human Breast Cancer Cell Line.
V-6 G. CITRO, L. D'Agna, and G. Zupi.
16:30 Studies on MDR Variants of Human Lung Carcinoma Lines.
V-7 M. CLYNES, A. Redmond, and E. Moran.

SUNDAY: 21 June **Regency F**

SESSION-IN-DEPTH

V Signal Transduction in Olfactory Receptors
Conveners: Roberto Revoltella, Instituto di Mutagenesi e Differenziamento, Italy and Jonathan Pevsner, Johns Hopkins University School of Medicine, USA

14:00 Olfactory Organ Culture In Vivo and In Vitro.
V-8 P. P. C. GRAZIADEI.
14:45 Peripheral Aspects of Odor Perception.
V-9 P. PELOSI.
15:30 Molecular Mechanisms of Olfactory Signal Transduction.
V-10 H. BREER, I. Boekhoff, J. Krieger, K. Raming, J. Strotmann, and E. Tareilus.
16:15 Regulation of Olfactory Neuron Gene Expression.
V-11 F. L. MARGOLIS.

SUNDAY: 21 June **Potomac VI**
16:00-18:00

PUBLICATION'S COMMITTEE MEETING

SUNDAY: 21 June **Arlington**
17:00-18:00

CELLULAR TOXICOLOGY COMMITTEE BUSINESS MEETING

SUNDAY: 21 June **Roosevelt**
18:30-20:00

STUDENT SOCIAL
Students are Encouraged to Attend

SUNDAY: 21 June **Potomac I-II**

WORKSHOP

I Insect Host-Pathogen and -Parasite Interactions In Vitro
Convener: Pauline O. Lawrence, University of Florida, USA

20:00 Use of a Cell-Free Medium to Determine the Source of a Parasitism-Specific Protein Found in the Hemolymph of a Fruit Fly Host.
W-1 P. O. LAWRENCE.
20:15 Embryonic Development of an Endoparasitoid, *Microplitis croceipes* (Hymenoptera: Braconidae) In Vitro.
W-2 S. FERKOVICH and H. Oberlander.
20:30 Infection of *Choristoneura fumiferana* (Lepidoptera: Tortricidae) Cell Lines with the Microsporidium *Pleistophora schubergi* Zwolfer.
W-3 S. S. SOHI and G. G. Wilson.
20:45 Gene Expression of a Baculovirus Recombinant in Permissive and Nonpermissive Insect Cells.
W-4 A. H. McINTOSH and J. J. GRASELA.

SUNDAY: 21 June **Potomac I-II**
22:00

INVERTEBRATE DIVISION BUSINESS MEETING

SUNDAY: 21 June **Regency E**

WORKSHOP

P Plant Germplasm Storage Technology
Convener: Phillip C. Stanwood, USDA-ARS, USA

Will the advent of new technologies mean that conventional seed repositories will be obsolete? Conventional and new approaches to the collection and storage of valuable plant germplasm will be discussed and evaluated with a view to utilization of the plant genetic resources available to us and the biological limitations to their exploitation.

20:00 Plant Germplasm Storage Technology.
W-5 P. C. STANWOOD.
20:30 Techniques for Clonal Germplasm Preservation.
W-6 B. M. REED.
21:00 Cryopreservation and Other Technologies for Long-Term Storage of Clonal Crop Germplasm.
W-7 L. E. TOWILL.
21:30 The Use of Tissue Culture Techniques in the Preservation of "Orthodox" and "Recalcitrant" Seeds.
W-8 H. W. PRITCHARD.

SUNDAY: 21 June **Regency A**

WORKSHOP
Co-sponsored by the European Tissue Culture Society

20:00 **V Interlab Projects and Data Bases**
Convener: Alan Doyle, European Tissue Culture Society

SUNDAY: 21 June **Exhibit Hall B**
21:00 **Set-Up Posters**

MONDAY: 22 June **Exhibit Hall B**

POSTER SESSION

8:00 a.m.-Midnight

Posters must removed from Exhibit Hall
Wednesday, 24 June, 15:00

Authors will be present Monday, 22 June	
Odd Numbers	13:00-14:00
Even Numbers	17:00-18:00

SEE LIST OF POSTERS ON PAGES 30-39

★ **LAST CHANCE** ★
Monday 12:00 noon
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MONDAY: 22 June **Regency E**

SESSION-IN-DEPTH

Sponsored by Costar Corporation

T In Vitro Systems for Drug Evaluation and Testing
Conveners: Hank Lane, Costar Corporation, USA and Per Artursson, Uppsala University, Sweden

7:30 T-9 Cooperation of Two Cell Types for the Assembly of sIgA in Culture.
J. P. KRAEHENBUHL, R. Hirt, and N. Jeanguenat.

8:05 T-10 Cultured Brain Microvessel Endothelial Cells: An In Vitro Model of the Blood Brain Barrier.
R. T. BORCHARDT.

8:40 T-11 Drug and Peptide Absorption in Human Intestinal Epithelial Cell Cultures.
P. ARTURSSON.

9:15 T-12 The Transport of Vitamin B_{12} Across Monolayers of Caco-2 Cells Grown on Permeable Supports.
I. F. HASSAN and M. Mackay.

9:50 T-13 Cultured Rabbit Tracheal Epithelial Cells: An In Vitro Model of Upper Respiratory Drug Penetration.
C. S. SCHASTEEN, S. R. Rapp, and B. T. Keller.

MONDAY: 22 June **Regency F**

SESSION-IN-DEPTH

JS (I & V) Regulation of Gene Expression
Convener: Edward M. Berger, Dartmouth College, USA

8:00 JS-1 Transcriptional Regulation of Pathogenesis by the Moloney Murine Leukemia Virus.
N. A. SPECK, S. Wang, I. Cherepennikova, Y. H. Hsiang, and D. H. Raulet.

8:40 JS-2 Regulation of Gene Expression by the Nuclear Hormone Receptor Superfamily.
D. D. MOORE.

9:20 JS-3 Regulation and Function of the *E75* Ecdysone-Inducible Gene of *Drosophila*.
W. A. SEGRAVES.

10:00 JS-4 Molecular Biology of Ecdysone and Juvenile Hormone Action.
E. M. BERGER.

MONDAY: 22 June **Regency A-B**

SESSION-IN-DEPTH

P Genetics and Biochemistry of Plant Regeneration

Conveners: Charles L. Armstrong, Monsanto Agricultural Company, USA and S. C. de Vries, Agricultural University Wageningen, The Netherlands

Genetic limits to plant regeneration hinder the full exploitation of tissue culture technology. Chromosomal localization of genes important for somatic embryogenesis could facilitate breeding for enhanced regenerability. Alternatively, improved understanding of the biochemical modulation of plant embryogenesis could lead to media manipulations which would allow efficient regeneration from any genotype. The two approaches complement each other--precise definition of the genetics of plant regeneration could help elucidate the underlying biochemical process. This session will focus on some recent developments in our understanding of the genetic and biochemical control of plant embryogenesis.

8:00 RFLP Analysis of Plant Regeneration in Maize.
P-5 C. L. ARMSTRONG, J. Romero-Severson, and T. K. Hodges.
8:30 Proteins Associated with the Induction of *Brassica napus* Microspore Embryogenesis.
P-6 J. H. G. CORDEWENER, R. Busink, Y. Nöllen, J. B. M. Custers, J. A. Traas, and J. J. M. Dons.
9:10 Developmental and Molecular Genetics of Embryogenesis in *Arabidopsis thaliana*.
P-7 D. W. MEINKE.
9:30 Secreted Proteins as Modulators of Plant Embryogenesis.
P-8 S. C. DE VRIES, T. Hendrick, A. J. de Jong, M. V. Hartog, E. A. Meijer, and A. van Kemmen.

MONDAY: 22 June **Washington A-B**

SESSION-IN-DEPTH

T Metabolic and Molecular Injury by Metals: Quantitation and Significance

Convener: Evelyn Tiffany-Castiglioni,Texas A&M University, USA

8:00 Introduction
8:10 Induction of Heat Shock Proteins (HSPs) in Sertoli Cells by Cadmium.
T-14 M. J. BRABEC, L. Batarseh, and M. J. Welsh.
8:45 Molecular Interactions of Lead in Cultured Astroglia.
T-15 E. TIFFANY-CASTIGLIONI.
9:20 Use of Isolated Hepatocytes and Organ Slices to Study In Vitro the Metabolic Injury by Metals.
T-16 F. GOETHALS, P. Buc-Calderon, and M. Roberfroid.
9:55 In Vitro Modulation of Human Lymphocytes and Monocytes by Metals.
T-17 D. A. LAWRENCE and J. M. Slavik.

MONDAY: 22 June **Regency F**

SESSION-IN-DEPTH

V Cancer--Viral Genes

Convener: Hilary Koprowski, The Wistar Institute, USA

8:30 A Molecular Cytogenetic Assessment of Structural and Numerical Aberrations Associated with Human Cancers.
V-12 J. W. GRAY, O. Kallioniemi, A. Kallionieni, M. Sakamoto, K. Matsumura, F. Waldman, and D. Pinkel.
9:10 TBA
V-13 M. LEPPERT.
9:50 TBA
V-14 C. CROCE.

MONDAY: 22 June **Exhibit Hall A**

EXHIBITS

Exhibits will be open

MONDAY: 10:00 a.m.-18:00 p.m.
TUESDAY: 10:00 a.m.-18:00 p.m.
WEDNESDAY: 10:00 a.m.-17:00 p.m.

MONDAY: 22 June **Regency A-B**

PLENARY SESSION

11:00 **U.S. Government Science Policy**
Convener: Elliot M. Levine, The Wistar Institute, USA

LIFETIME ACHIEVEMENT AWARDS
Recipients to be Announced

KEYNOTE ADDRESS

BERNADINE P. HEALY
DIRECTOR of the NIH

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MONDAY: 22 June **Potomac III**
12:00-14:00

PLANT EDITORIAL BOARD COMMITTEE MEETING

MONDAY: 22 June **Potomac I-II**

SESSION-IN-DEPTH

I Gene Transfer in Cultured *Drosophila* Cells
Conveners: Guy Echalier and M. Best Belpomme, Université P.-et-M.-Curie, France

14:00 Variability of Transfection Efficiency Among *Drosophila* Cell Lines.
I-5 G. ECHALIER.
14:30 Functional Studies of Ecdysone Response Elements by Transfection of *Drosophila* Kc Cells.
I-6 L. CHERBAS.
15:00 Regulation by *Drosophila* Segmentation Genes In Vivo and in Tissue Culture Cells.
I-7 H. JÄCKLE, F. Sauer, M. Hoch, and N. Gerwin.
15:30 Co-Activators Associated with TATA-Binding Protein Mediate Transcriptional Activation in *Drosophila*.
I-8 B. D. DYNLACHT, T. Hoey, R. Weinzierl, and R. Tijian.
16:00 Expression and Regulation of 1731, a *Drosophila* Retrotransposon.
I-9 M. BEST-BELPOMME, P. Ziarczyk, C. Coulondre, M. Y. Kim, C. Maisonhaute, E. Nahon, J. M. Saucier, S. Simonart-Codani, and F. Fourcade-Peronnet.

MONDAY: 22 June **Regency E**

SESSION-IN-DEPTH

P New Approaches to Plant Transformation

Convener: Ted Klein, DuPont Agricultural Products, USA and Mark Van Montague, University of Gent, Belgium

Foreign genes can be transferred to a wide range of plant species. However, the ease with which gene transfer can be accomplished varies between species and even between genotypes of the same species. Therefore, the development of gene transfer technologies remains an important component of research programs directed toward studying gene expression and improving crops by recombinant DNA techniques. Currently, there are three major categories of gene transfer technologies. These are 1) gene transfer by *Agrobacterium*, 2) gene transfer to regenerable protoplasts, and 3) direct gene transfer to intact cells and tissues by microprojectiles. Efforts in a number of labs are directed at refining and improving the efficiency of the existing systems. In addition, researchers are investigating the factors that limit the host range of *Agrobacterium* and are searching for new methods for direct delivery of DNA into intact tissues (i.e., silica carbide fibres, electroporation).

14:00 Introduction

14:10 Transformation of Recalcitrant Crops Using Electric Discharge Particle Acceleration.
P-10 P. CHRISTOU.

14:40 The Combination of Microprojectile Bombardment and *Agrobacterium tumefaciens* Improves Plant Transformation Frequencies.
P-9 D. L. BIDNEY, C. J. Scelonge, and J. Malone-Schoneberg.

15:10 Silicon Carbide Fiber-Mediated Transformation of Plant Tissue Cultures.
P-13 D. A. SOMERS and H. F. Kaeppler.

15:40 Genetic Manipulation of *Agrobacterium tumefaciens* for the Transformation of Recalcitrant Plant Species.
P-11 S. B. GELVIN and C.-N. Liu.

16:10 Electroporation of Intact Tissues.
P-12 M. VAN MONTAGUE.

MONDAY: 22 June **Regency F**

SESSION-IN-DEPTH

P & V Extracellular Matrices and Effects on Differentiation and Proliferation

Conveners: Wallace L. McKeehan, W. Alton Jones Cell Science Center, USA and Norma Trolinder, USDA-ARS, USA

Extracellular components such as the plant cell wall and the extracellular matrix have been shown to play an important role in the regulation of cell organization. This session will look at the role of the extracellular matrix on cell organization and mechanisms whereby cell organization is mediated by extracellular components.

14:00 Biogenesis of Secondary Cell Walls of Flowering Plants.
JS-5 C. H. HAIGLER.

14:45 Structural Models of Primary Cell Walls of Flowering Plants.
JS-6 N. C. CARPITA and D. M. Gibeaut.

15:30 Extracellular Matrix and the Control of Cell Migration.
JS-7 S. L. SCHOR, L. Ellis, A. M. Schor, and A. M. Grey.

16:15 Direct Interaction of Heparin with Heparin-Binding (Fibroblast) Growth Factor (HBGF)-Receptor Mediates Ligand Binding.
JS-8 M. KAN, J. Hou, J. Xu, E. Shi, F. Wang, G. Yan, and W. L. McKeehan.

MONDAY: 22 June **Washington A-B**

SESSION-IN-DEPTH

T Tissue Culture Models for Lung Toxicity
Convener: Roger A. Coulombe, Jr., Utah State University, USA

14:00 Introduction
14:10 Tracheal Explant Cultures as Models for Lung Carcinogenesis.
T-18 R. A. COULOMBE, JR.
14:50 Large Airway Cell and Organ Culture Systems for Exposure to Oxidant Gasses.
T-19 D. W. WILSON, R. Wu, and B. Tarkington.
15:30 Isolation and Culture of Small Airway Cells for Experiments in Selective Toxicity and Metabolism.
T-20 C. PLOPPER, C. Chichester, L. Stewart, A. Pang, A. Chang, and W. Cardoso.
16:10 In Vitro Hamster/Human Clara Cell Differentiation for Lung Toxicology.
T-21 M. EMURA, A. Ochiai, M. Riebe-Imre, G. Singh, S. L. Katyal, J. Knebel, U. Mohr, D. L. Dungworth, J. Jacob, and G. Grimmer.

MONDAY: 22 June **Regency C-D**

SESSION-IN-DEPTH

V Hematopoietic Stem Cells
Conveners: Sandro Eridani, National Research Council, Italy and Keith Humphries, The Terry Fox Laboratory for Hematology/Oncology, Canada

14:00 Identification of Human Haemopoietic Stem Cells (HSC).
V-15 S. ERIDANI and R. Paleari.
14:15 Expression of Genes in Murine Bone Marrow Fractions Enriched for Pluripotent Haemopoietic Stem Cells.
V-16 J. W. M. VISSER, M. G. C. Hogeweg, H. Rozemuller, A. Belyavsky, and K. Rajewsky.
14:45 The Generation of Human Colony-Forming Cells (CFC) and the Expansion of Hematopoiesis In Vitro is Dependent on the Presence of Stem Cell Factor (SCF).
V-17 A. R. MIGLIACCIO, G. Migliaccio, G. C. Mancini, K. Zsebo, and J. W. Adamson.
15:15 Hematopoietic Differentiation of Embryonic Stem (ES) Cells in Culture.
V-18 M. Kennedy, M. Wiles, and G. KELLER.
15:45 Characterization of a Stem Cell Inhibitor: Pre-Clinical Studies.
V-19 D. J. Dunlop, E. G. Wright, S. Lorimore, G. J. Graham, T. Holyoake, D. J. Kerr, S. D. Wolpe, and I. B. PRAGNELL.
16:15 TBA
V-20 K. HUMPHRIES.

MONDAY: 22 June **Potomac III**
15:00-17:00

CELL CULTURE STANDARDIZATION COMMITTEE MEETING

17:15-19:00 **VERTEBRATE DIVISION BUSINESS MEETING** **Potomac IV**

18:00-20:00 JAPANESE TISSUE CULTURE ASSOCIATION COMMITTEE MEETING Arlington

MONDAY: 22 June **Potomac I-II**

WORKSHOP

I Invertebrate Cellular Immunity: In Vitro Aspects

Conveners: Robert S. Anderson, Chesapeake Biological Laboratory, USA and G. R. Vasta, Center for Marine Biotechnology, University of Maryland, USA

19:00 Time-Lapse Cinematography of Hemocytes of the Clam, *Mercenaria mercenaria.*
W-9 A. F. EBLE.
19:20 Influence of the Oyster Environment on the Activities of Oyster Hemocytes in Primary Culture.
W-10 W. S. FISHER.
19:40 Effect of Xenobiotic.
DANIELS.
20:00 TBA
YOSHINO.
20:20 In Vitro Interactions Between Molluscan Hemocytes and the Parasitic Protozoan *Haplosporidium nelsoni* (MSX).
W-11 S. E. FORD and K. A. Alcox.
20:40 Infection of Oysters with *Perkinsus marinus* Enhances Chemiluminescence by Hemocytes.
W-12 R. S. ANDERSON, K. T. Paynter, and E. M. Burreson.
21:00 Carbohydrate Binding Proteins.
G. R. VASTA.
21:20 Survival and Changes in Pharyngeal Explants of a Solitary Tunicate.
W-13 T. Sawada, E. L. COOPER, and J. Zhang.
21:40 The Inducible Humoral Immune Response in the American Cockroach: Site of Synthesis of the Immune Proteins.
W-14 R. D. KARP and S. Ewashinka.

MONDAY: 22 June **Regency A-B**

WORKSHOP

P Bioreactor Technology

Convener: Roy Harrell, University of Florida, USA

Will bioreactors soon replace the Petri dish and the Erlenmeyer flask in most labs for routine production of cells and somatic embryos? Recent advances in large-scale culture of specialized cells in batch and semi-continuous cultures indicate that factors such as gas exchange and micronutrient toxicity that have not previously been considered limiting must be taken into account when scaling up from the Petri dish and the Erlenmeyer flask. Highly refined bioreactors developed with bacteria culture in mind are proving to be inadequate for plant use and new vessel designs are helping to redefine the physiological parameters of cell culture but also the overall process of micropropagation.

19:00 W-15a Engineering Experiences with Bioreactor Scale-Up of Somatic Embryogenesis.
R. HARRELL, M. Bienick, and D. J. Cantliffe. (See Abstract #W-24)
19:30 Low Cost Air Lift Bioreactors for Multiple Comparisons of Growth Conditions.
W-15b N. L. TROLINDER, J. Parker, R. D. Allen, and C. Ashcroft.

MONDAY: 22 June **Washington B**

WORKSHOP

Co-sponsored by the European Tissue Culture Society

19:00 **V Paracrine Growth Control**

Convener: Norbert E. Fusenig, Institut fur Biochemie, Germany

MONDAY: 22 June **Potomac V-VI**

WORKSHOP

Sponsored by Microbiologial Associates, Inc.

20:00 **T Latest Techniques Applicable to In Vitro Toxicology**

Conveners: Rodger D. Curren and John W. Harbell, Microbiological Associates, Inc., USA

The workshop will be introduced by a short overview of the state-of-the-art of in vitro toxicological methods. Over a dozen assay systems will be demonstrated with opportunity for attendees to observe, first-hand, the operation of the novel instrumentation and required laboratory techniques.

20:00 **PLANT DIVISION BUSINESS MEETING** **Regency E**

20:30 **PLANT DIVISION SOCIAL** **Regency E**

TUESDAY: 23 June **Hotel Restaurant**

7:00

NCATS CELL MATES BREAKFAST

7:30 **MIDWEST BRANCH BUSINESS MEETING** **Potomac IV**

TUESDAY: 23 June **Exhibit Hall B**

POSTER SESSION

8:00 a.m.-Midnight

Posters must removed from Exhibit Hall
Wednesday, 24 June, 15:00

Authors will be present Tuesday, 23 June	
Even Numbers	13:00-14:00
Odd Numbers	17:00-18:00

SEE LIST OF POSTERS ON PAGES 30-39

TUESDAY: 23 June **Regency E-F**

PLENARY SESSION

Gene Identification
Convener: Daniel C. W. Brown, Plant Research Centre, Canada

8:00 An Efficient Method for Isolating and Mapping Human Genes.
PS-5 R. KUCHERLAPATI, A. Skoultchi, R. Das Gupta, S. Parimoo, and S. Weissman.
8:30 Transposable Elements as Tools to Isolate and Characterize Genes from *Antirrhinum majus*.
PS-6 H. SAEDLER.
9:00 Cloning of a New Gene Concerning Cell Proliferation.
PS-7 T. MARUNOUCHI and H. Hosoya.

TUESDAY: 23 June **Exhibit Hall A**

EXHIBITS

Exhibits will be open

MONDAY:	10:00 a.m.-18:00 p.m.
TUESDAY:	10:00 a.m.-18:00 p.m.
WEDNESDAY:	10:00 a.m.-17:00 p.m.

10:00-14:00 **LONG RANGE PLANNING COMMITTEE MEETING** **Potomac V**

TUESDAY: 23 June **Potomac I-II**

CONTRIBUTED PAPERS

I Culture Conditions for Marine Invertebrate and Insect Cells
Convener: Ann Fallon, University of Minnesota, USA

10:30 Development of Primary Culture of Epidermal Cells from the Penaeid Shrimp, *Penaeus vannamei*.
I-1001 J.-Y. TOULLEC and P. Porcheron.
10:45 Primary Culture of Lymphoid and Nerve Cells from *Penaeus stylirostris* and *Penaeus vannamei*.
I-1002 E. C. B. Nadala, Y. Lu, and P. C. LOH.
11:00 Discovery of EGF-Like Factor in Mollusks.
I-1003 N. A. ODINTSOVA and D. A. Korchagina.

11:15 Initiation and Promotion of Hematopoietic Neoplasia in Soft Shell Clams Exposed to Natural Sediments.
I-1004 D. F. LEAVITT, D. Miosky, B. A. Lancaster, A. C. Craig, C. L. Reinisch, and J. McDowell Capuzzo.
11:30 Utilization of Carbohydrates and Accumulation of Lactate in *Spodoptera frugiperda* Insect Cell Cultures.
I-1005 A. T. NAHAPETIAN, T. A. Trivits, J. T. Pepe, and J. R. Orton.
11:45 Adaptation of *Orgyia leucostigma* (Lepidoptera: Lymantriidae) IPRI-OL-12 Cells to Serum-Free Media.
I-1006 S. S. SOHI, G. F. Caputo, and W. Lalouette.
12:00 Viral Pesticides: Production in Serum-Free Insect Cell Culture.
I-1007 S. A. WEISS, G. P. Godwin, W. G. Whitford, E. M. Dougherty, and G. F. Rohrman.

TUESDAY: 23 June **Regency E**

CONTRIBUTED PAPERS

P Plant Secondary Products

Conveners: Pamela J. Weathers, Worcester Polytechnic Institute, USA and Abraham Rubluo, Institute of Biology, Mexico

10:30 In Vitro Production of the Potential Industrial Compound Piquerol from *Piqueria trinervia*.
P-1018 A. RUBLUO, A. Flores, and M. Jiménez.
10:45 The Effects of Immobilization, Exogenous Enzymes and Precursors on the Production of Artemisinin by Cell Culture of *Artemisia annua*.
P-1019 P. K. CHEN and C. Lukonis.
11:00 A Comparison of the Monoterpenoid Constituents of Rosemary Plants Derived from Stem Cuttings and from Leaf Segment Cultures.
P-1020 A. A. TAWFIK and P. E. Read.
11:15 Biosynthesis of Lower Terpenes in the Plant Cultured Cells.
P-1021 K. NABETA and M. Sakurai.
11:30 Development of Callus and Cell Suspension Cultures for Taxol Production.
P-1022 E. R. M. WICKREMESINHE and R. N. Arteca.
11:45 Cardiac Glycosides in Crown Galls of *Digitalis lanata*.
P-1023 M. LUCKNER, W. Pinkwart, W. Kreis, and B. Diettrich.
12:00 Differentiation with Regards to Anthocyanin Production in Grape (*Vitis vinifera*) Cell Suspension.
P-1024 F. CORMIER and C. B. Do.
12:15 Artemisinin Production by *Artemesia annua*: Environmental Factors and Transformation.
P-1025 P. J. WEATHERS, R. D. Cheetham, S. Kovacs, A. Hoyen, T. Canty, and G. Auger.

TUESDAY: 23 June **Regency F**

CONTRIBUTED PAPERS

P Plant Tissue Culture

Conveners: Kris Pruski, Alberta Tree Nursery and Horticulture Centre, Canada and Denise M. Seliskar, University of Delaware, USA

10:30 Tissue Culture and Regeneration of Coastal Dune Grasses.
P-1026 D. M. SELISKAR.
10:45 Somatic Embryogenesis in the Halophyte *Sporobolus virginicus* (L.) Kunth.
P-1027 J. D. RAO, X. Li, and J. L. Gallagher.
11:00 Preparation of Protoplasts of Halophytes and the Evaluation of Salinity Tolerance as a Natural Fusion Marker in *Kosteletskya virginica* and *Sporobolus virginicus* (L) Kunth.
P-1028 X. LI, J. D. Rao, and J. L. Gallagher.
11:15 Somatic Embryogenesis on Immature Cotyledons of Watermelon.
P-1029 M. E. COMPTON and D. J. Gray.
11:30 Inheritance of Green Plant Production in Wheat Anther Culture.
P-1030 H. ZHOU and C. K. Konzak.

11:45 The Effect of Epi-24 Brassinolide on Callus and Root Formation in Saskatoon (*Amelanchier alnifolia* Nutt.) Microcuttings.
P-1031 K. PRUSKI, T. Lewis, and M. Mirza.
12:00 Desiccation and Cryopreservation of Embryo Axes of *Quercus* sp.
P-1032 V. C. PENCE.
12:15 High Frequency Adventitious Shoot Regeneration and Transient Gene Expression in Pea Cotyledon Explants.
P-1033 S. ÖZCAN, M. Barghchi, N. Bate, S. Firek, D. Twell, and J. Draper.
12:30 Studies on Tissue Culture of *Eucommia ulmoides* Oliv.
P-1034 Y. TODA and Y. Nakazawa.
12:45 In Vitro Shoot Formation of Cacti Species in Response to Cytokinins and Auxin.
P-1035 M. A. BUSTAMANTE and L. G. Tovar.

TUESDAY: 23 June **Washington A-B**

CONTRIBUTED PAPERS

T Toxicology II
Convener: Rodger D. Curren, Microbiological Associates, Inc., USA

10:30 Cytotoxic Effects of FK506 on Cultured Human Renal Proximal Tubule Cells.
T-1009 M. ATCHERSON, K. Baghelai, and A. Trifillis.
10:45 Human Corneal Epithelial Primary Cultures and Immortalized Cell Lines: In Vitro Model for Ocular Studies.
T-1010 C. R. KAHN, I. H. Lee, and J. S. Rhim.
11:00 Cytotoxicity Evaluation from the Viewpoint of Cell Recovery.
T-1011 K. IMAI and M. Nakamura.
11:15 In Vitro Cytotoxicity Testing: Biological and Statistical Significance.
T-1012 F. A. BARILE, S. Arjun, and D. Hopkinson.
11:30 Neutral Red (NR) Assay for Potency Study of Chemicals in Common Use.
T-1013 E. BORENFREUND and H. Babich.
11:45 A 3-Dimensional Human Skin Culture System Used in Toxicity Testing of Topically Applied Test Agents.
T-1014 D. TRIGLIA, T. Donnelly, I. Kidd, and S. Sherard Braa.
12:00 Comparison of the Results from 100 Different Cytotoxicity Assays of the First 10 MEIC Chemicals.
T-1015 B. EKWALL, F. Barile, H. Bjerregaard, C. Chesne, R. Clothier, P. Dierickx, M. Ferro, G. Fiskesjö, L. Garza-Ocanas, M. J. Gómez-Lechón, M. Gülden, B. Isomaa, T. Jacobsen, U. Kristen, M. Kunimoto, S. Kärenlampi, L. Lewan, M. Nordin, G. Persoone, T. Sawyer, H. Seibert, R. Shrivastava, A. Stammati, M. Tingleff-Skaanild, D. Triglia, C. Tyson, and E. Walum.

TUESDAY: 23 June **Regency A-B**

CONTRIBUTED PAPERS

V Cell Aging and Immortalization
Convener: Robert Dell'Orco, The Samuel Roberts Noble Foundation, Inc., USA

10:30 Extracellular Matrix (ECM) Gene Expression by Fibroblasts In Vivo and In Vitro.
V-1017 S. A. BRUCE, A. M. Choi, M. H. Swee, and S. F. Deamond.
10:45 Proliferating Cell Nuclear Antigen Expression in Aging Human Diploid Cells.
V-1018 C. A. Stewart and R. T. DELL'ORCO.
11:00 Expression of Prohibitin in Young and Old Human Diploid Fibroblasts.
V-1019 J. K. McCLUNG, X.-T. Liu, L. S. Walker, R. L. King, C. A. Stewart, and R. T. Dell'Orco.

11:15 Effect of Human Chromosome 1 on the Proliferative Potential of Various Immortal Human Cell Lines.
V-1020 P. J. HENSLER, L. A. Annab, J. C. Barrett, and O. M. Pereira-Smith.
11:30 Expression of Genes Located on Human Chromosome 3 in Quiescent and Proliferating Fibroblast Line WI-38.
V-1021 Z. MARCSEK, E. R. Zabarovsky, Cs. Kiss, Z. Bori, and M. Kucsera.
11:45 Cooperativiity of SV40 T Antigen and *Ras* in Progressive Stages of Transformation of Human Fibroblasts.
V-1022 J. A. WHITE, S. G. Carter, H. L. Ozer, and A. L. Boyd.
12:00 Immortalization of Normal Human Fibroblasts by Treatment with 4-Nitroquinoline 1-Oxide (4NQO).
V-1023 M. NAMBA, L. Bai, M. Miyazaki, H. Mizusawa, and M. Honma.
12:15 Expression of Simian Virus 40 Early Genetic Region in Human Endothelial Cells.
V-1024 O. HOHENWARTER, E. Zinser, C. Schmatz, and H. Katinger.

TUESDAY: 23 June **Potomac I-II**

SESSION-IN-DEPTH

I Invertebrate Tissue Culture as a Tool to Study Insect-Transmitted Viruses
Convener: Hans Koblet, University of Berne, Switzerland

14:00 Viral Evolution and Insects as a Possible Virological Turning Table.
I-10 H. KOBLET.
14:35 Insect Transmitted Plant Viruses.
I-11 R. CREAMER.
15:10 The Study of Alpha Togaviruses in Cultured Mosquito Cells.
I-12 V. STOLLAR.
15:45 Insect Transmitted Vertebrate Viruses: Flaviviridae.
I-13 G. LUDWIG.
16:20 Insect Transmitted Vertebrate Viruses: Bunyaviridae.
I-14 C. S. SCHMALJOHN.

TUESDAY: 23 June **Regency E**

SESSION-IN-DEPTH

P Exploitation of Plant Cell Culture Variants
Convener: Frederick Meins, Jr., Friedrich Miescher Institute, Switzerland and Jack Widholm, University of Illinois, USA

Plant cells in culture undergo extensive genetic and epigenetic variation. This session deals with the nature of this variation and its application for improving crop plants and producing biologically active secondary products.

14:00 On the Nature of Cell Heritable Variation in Culture.
P-14 F. MEINS, JR. and M. Seldran.
14:45 Implications of DNA Methylation Alterations.
P-15 R. L. PHILLIPS and S. M. Kaeppler.
15:30 In Vitro Selection for Plant Improvement.
P-16 J. M. WIDHOLM.
16:15 Establishment of Cell Cultures of *Taxus brevifolia* (Pacific Yew) for Taxol Production.
P-17 D. M. GIBSON and R. E. B. Ketchum.

TUESDAY: 23 June **Regency F**

SESSION-IN-DEPTH

P Micropropagation for Biotechnology and Agriculture
Convener: Irwin Chu, Twyford International, Inc., USA

Micropropagation is a new business segment created by advanced plant tissue culture production technology. Products can be produced through micropropagation faster than conventional methods and the quality of the product is good, uniform and free of pathogen. Numerous new products have also been created and introduced into the market place. Recently, micropropagation has become a stable and profitable business, especially inthe horticultural industry and is rapidly expanding its application to agriculture, plantation crops and forestry. This session will cover the entire story of past, present and future as well as the technology, production, market and business areas. The four speakers represent three companies which account for 80% of the North American market.

14:00 Introduction
14:10 The History of the Micropropagation Business.
P-18 R. STRODE.
14:50 Current Micropropagation Production System.
P-19 R. D. HARTMAN.
15:30 Current Application of Micropropagation.
P-20 G. R. HENNEN.
16:10 The Future of the Micropropagation Business.
P-21 I. CHU.
16:40 Discussion and Comments

TUESDAY: 23 June **Regency C**

SESSION-IN-DEPTH

T Cell and Tissue Models for the Study of Mycotoxins
Conveners: T. Rick Irvin, Louisiana State University, USA and Valery Smirnov, Kiev State University, Russia

14:00 Use of Hydra and Rodent Embryo Cultures for the Study of Teratogenic Mycotoxins.
T-22 T. D. PHILLIPS.
14:40 Development and Application of Mouse Blastocysts Culture Systems to Evaluate the Prenatal Toxicity of Mycotoxins.
T-23 T. R. IRVIN.
15:20 Action of Aflatoxin B_1 in Lung Airway Cultures.
T-24 R. A. COULOMBE, JR.
16:00 Cell and Tissue Culture Evaluation of Mycotoxins Operative in Human and Animal Disease.
T-25 V. SMIRNOV.

TUESDAY: 23 June **Washington A-B**

SESSION-IN-DEPTH

T Cell Culture Systems to Evaluate Chemically-Induced Teratogenesis and Prenatal Toxicology
Convener: John E. Martin, Louisiana State University, USA

14:00 Introduction
14:10 Primary Embryo Cell Cultures: Utility in Developmental Toxicity Studies.
T-26 E. M. FAUSTMAN and S. Whittaker.

14:45 Mechanisms in Teratogenesis Studied with Whole Rat Embryos in Culture.
T-27 T. J. FLYNN.
15:20 Postimplantation Embryo Culture Systems for Mechanistic Studies of Prenatal Toxins.
T-28 T. R. IRVIN.
15:55 In Vitro Embryotoxicity Test Using Blastocyst Derived Euploid Embryonal Stem (ES) Cells of the Mouse.
T-29 H. SPIELMANN, G. Klein, A. Pöting, and R. Vogel.

TUESDAY: 23 June **Regency A**

SESSION-IN-DEPTH

V Hepatic Cell Cultures

Conveners: Christaine Guguen-Guillouzo, Hopital Pontchaillou, France and Gretchen J. Darlington, Baylor College of Medicine, USA

14:00 Hepatic Cell Lineages.
V-21 S. S. THORGEIRSSON.
14:35 Receptor Phenotype Underlies Differential Response of Hepatocytes and Non-Parenchymal Cells to Heparin-Binding Fibroblast Growth Factor Type 1 (aFGF) and Type 2 (bFGF).
V-22 W. L. McKEEHAN, M. Kan, G. Yan, J. Xu, M. Nakahara, and J. Hou.
15:10 Biomatrix and Liver Cell Differentiation.
V-23 D. M. BISSELL.
15:45 Plasma Membrane Proteins Involved in Cell-Cell Contact Mediated Regulations of Hepatocyte Functions.
V-24 C. GUGUEN-GUILLOUZO and A. Corlu.
16:20 Isolation of Differentiated Immortal Hepatocyte Lines from Transgenic Mice.
V-25 D. PAUL and B. Hoffmann.

TUESDAY: 23 June **Regency D**

SESSION-IN-DEPTH

V Tissue Culture of Killer Cells

Convener: Daniela Santoli, The Wistar Institute, USA

14:00 Generation of Human Cytotoxic Lymphocytes Requires Both IFN-γ and TNF-α-Mediated Signals.
V-26 F. NOVELLI, M. Giovarelli, S. Vai, F. DiPierro, M. Zucca, G. Garotta, and G. Forni.
14:45 Stable Expression of Tumoricidal Activity by IL-2-Dependent Human Leukemic T Cell Lines.
V-27 D. SANTOLI and A. Cesano.
15:30 Transfer of $CD8^+$ Cytomegalovirus (CMV)-Specific Immunity After Bone Marrow Transplant by Adoptive Immunotherapy with T Cell Clones.
V-28 S. R. RIDDELL, K. Watanabe, J. Goodrich, C. R. Li, M. Agha, and P. D. Greenberg.
16:15 Molecular Events Mediating Activation of Cytotoxic Cells.
V-29 D. COLLAVO, P. Zanovello, A. Rosato, A. Zambon, F. Pollis, C. Sorio, and A. Berton.

TUESDAY: 23 June **Potomac IV**
17:00-19:00

PCTOC EDITORIAL BOARD MEETING

TUESDAY: 23 June **Regency A**

WORKSHOP

P Influence of Physical Microenvironment on Plant Growth In Vitro
Convener: Mary Ann Lila Smith, University of Illinois, USA

The physical microenvironment, although often underestimated or ignored, is capable of exerting a tremendous influence on plant productivity and quality in microculture. The physical influences on culture growth [including light source (quality/quantity/location), temperature, gelling agent or support, vessel type, etc.] can interact with chemical or plant factors to influence plant development, or each factor can work in concert with other physical factors. This workshop will specifically explore the contributions of the physical support (gel strength, gelling agent, other supports for plant growth on liqiuid media) as they influence water availability in vitro, and the quality of micropropagated palnts. In addition, the placement and intensity of illumination sources, light quality, and the effects of light interaction with culture components will be explored.

19:00 The Media are not What They Seem: Photochemical Changes in the Composition of Plant Tissue Culture Media.
W-16 R. P. HANGARTER.
19:30 TBA
K. TORRES.
20:00 Environmental Effect and Environmental Control in Plant Tissue Culture.
W-17 T. KOZAI and K. Fujiwara.
20:30 Complex Effects of Physical Support on In Vitro Growth.
W-18 M. A. L. SMITH and L. A. Spomer.

TUESDAY: 23 June **Potomac I-II**

WORKSHOP

I Invertebrate Neoplasia: Initiation and Promotion Mechanisms
Conveners: Carol Reinisch, Tufts University School of Veterinary Medicine, USA and Ralph Elston, Battelle Marine Sciences Laboratory, USA

19:00 E. Twomey, M. Mulcahy, I. Sunila, S. McLaughlin, E. Gateff, M. Faisal, F. Hetrick, N. Blake, A. Marsh, G. Gardner, C. Reinisch, D. Leavitt, A. Farley, C. Dawe, N. A. Odintsova, D. A. Korchagina, E. Peters, K. Krieg, and A. Shearn.

TUESDAY: 23 June **Regency B**

WORKSHOP

P Synchronization of Cell Division and the Mechanisms of the Cell Cycle in Higher Plant Cells
Convener: Atsushi Komamine, Tohoku University, Japan

This workshop will explore recent advances on methods of synchronization of cell division in suspension cultures and protoplast cultures, and on physiological, biochemical and molecular aspects of mechanisms of the cell cycle in higher plants.

19:00 Expression of *Par* Genes During the Transition From G_0 to S Phase in Tobacco Mesophyll Protoplasts.
W-19 T. NAGATA and Y. Takahashi.
19:30 Synchronized Plant Cell Cultures as a Means to Study the Temporal Linkage of Cell Cycle Events.
W-20 M. PFOSSER, H. Hirt, E. Heberle-Bors, and R. Kandeler.
20:00 Cell Cycle Initiation and Progression in Cultured Protoplasts Derived from Differentiating and Differentiated Plant Cells.
W-21 W. WERNICKE, B. Holzhäuer, and G. Jung.

20:30 Components of the Cell Cycle Control in Cultured Plant Cells.
W-22 D. DUDITS, L. Bakó, Z. Magyar, J. Györgyey, F. Felföldi, T. Kapros, M. Deák, and D. Dedeoglu.
21:00 Gene Expression During the Cell Cycle in the Synchronized Cultures of *Catharanthus roseus* Cells.
W-23 A. KOMAMINE, M. Ito, H. Kodama, N. Ohnishi, and H. Hashimoto.

TUESDAY: 23 June **Regency C**

WORKSHOP

19:00 **T In Vitro Fertilization and Embryo Transfer Technology: Applications to Cellular Toxicology**
Convener: Pauline Cisneros, University of Texas Medical Center, USA

TUESDAY: 23 June **Regency D**

WORKSHOP

V Recent Advances in Fluorescent Assays for Cell and Tissue Culture
Convener: Richard Alcorn, Millipore Corporation, USA

19:00 Overview of Emerging Fluorescent Assays for Multiwell Plate Readers Including Cell Adhesion and Gene Expression.
R. ALCORN.
Analysis of Human Skin Equivalent.
R. VAN BUSKIRK
Cell Proliferation and DNA Quantitation Using Hoechst 33258.
A. PITT.
Determination of Cell Viability and Cytotoxicity.
P. L. MOORE.

WEDNESDAY: 24 June **Exhibit Hall B**

POSTER SESSION

8:00 a.m.-Midnight

Posters must removed from Exhibit Hall
Wednesday, 24 June, 15:00

SEE LIST OF POSTERS ON PAGES 30-39

WEDNESDAY: 24 June **Regency E-F**

PLENARY SESSION

P Transcriptional Control of Gene Expression

Convener: Ann Fallon, University of Minnesota, USA

8:00 Global and Local Regulation of Pattern Formation During Insect Development.
PS-8 S. B. CARROLL.

8:30 Transcriptional Regulation by Pou Homeodomain Proteins.
PS-9 W. HERR, R. Aurora, M. Cleary, G. Das, J.-S. Lai, S. Stern, M. Tanaka, W. Thomann, K. Visvanathan, and A. Wilson.

9:00 Transcriptional Control of Gene Expression in Plants.
PS-10 G. GALAU.

WEDNESDAY: 24 June **Exhibit Hall A**

EXHIBITS

Exhibits will be open

MONDAY: 10:00 a.m.-18:00 p.m.
TUESDAY: 10:00 a.m.-18:00 p.m.
WEDNESDAY: 10:00 a.m.-17:00 p.m.

WEDNESDAY: 24 June **Potomac I-II**

CONTRIBUTED PAPERS

I Invertebrate Cell Culture

Convener: Dwight Lynn, USDA-ARS, USA

10:00 Ecdysteroid-Stimulated Differentiation in a Lepidopteran Cell Line Derived from Imaginal Discs.
I-1008 P. PORCHERON and H. Oberlander.

10:15 In Vitro Culturing of the Verson's Gland of *Manduca sexta*, and the Role of 20-Hydroxyecdysone in Cell Growth and Pupal Commitment.
I-1009 K. L. HORWATH.

10:30 Correlation of Fat Body Histochemistry and Cell Morphology in Primary Cultures to Developmental Stages of *Manduca sexta* and *Tenebrio molitor*.
I-1010 C. M. EASTON and K. L. Horwath.

10:45 Survival Response of TN-368 Lepidopteran Cells to Psoralens and UVA Light.
I-1011 T. M. KOVAL and D. L. Suppes.

11:00 Stable Transfection of Mosquito Cells with a DNA Construct Containing the Dihydrofolate Reductase Gene.
I-1012 A. M. FALLON, F. A. Shotkoski, and Y.-J. Park.

11:15 Multiplication of a Baculovirus of *Malacosoma disstria* (Lepidoptera: Lasiocampidae) in a Homologous Cell Line.
I-1013 S. S. SOHI and B. J. Cook.

11:30 Cell Lines Used in Baculovirus Expression Vector Selection.
I-1014 J. E. MARUNIAK, A. Garcia-Canedo, and J. Rodrigues.

WEDNESDAY: 24 June **Regency E**

CONTRIBUTED PAPERS

P Plant Morphogenesis

Conveners: Dennis Gray, University of Florida, USA and P. K. Saxena, University of Guelph, Canada

10:00 In Vitro Regeneration of Grain Legumes: A Novel Approach.
P-1036 K. A. Malik and P. K. SAXENA.

10:15 Embryogenic Cultures from Various Explants of Soybean.
P-1037 P. S. KAHLON and S. M. Bhatti.

10:30 Artificial Seeds of Conifers.
P-1038 S. M. ATTREE and L. C. Fowke.

10:45 Tomato Fruit Aroma Compounds and Sugars: Further Evidence of the Altered Developmental Program of In Vitro Cultured Tomato Calyx.
P-1039 B. K. ISHIDA, E. A. Baldwin, R. G. Buttery, L. C. Ling, and G. H. Robertson.

11:00 Optimization of Plant Regeneration from Callus Cultures of *Gladiolus*.
P-1040 K. KAMO and M. Dorak.

11:15 Effects of Cytokinins, Genotype and Other Factors on Somatic Embryogenesis from Cotyledons of *Cucumis melo*.
P-1041 D. J. GRAY, D. W. McColley, and M. E. Compton.

11:30 Plant Regeneration and Somaclonal Variation in the Leaf Segment Culture of *Rumex acetosa*.
P-1042 J. W. BANG and M. K. Lee.

11:45 Triploid Plantlets from the Endosperm Culture of Some Euphorbiaceae.
P-1043 N. SYED ABBAS ALI.

12:00 Embryogenesis from Non-Juvenile Norway Spruce (*Picea abies*).
P-1044 R. J. WESTCOTT.

WEDNESDAY: 24 June **Regency F**

CONTRIBUTED PAPERS

P Plant Transformation

Conveners: Ken Kasha, University of Guelph, Canada

10:00 Transformation of Barley (*Hordeum vulgare* L.) Microspores.
P-1045 K. J. KASHA, A. Ziauddin, and E. Simion.

10:15 Development of the Particle Inflow Gun for Studies on Genetic Transformation of Plant Cells.
P-1046 J. J. FINER, P. Vain, M. W. Jones, and M. D. McMullen.

10:30 Bacteria and Yeast Cells as Projectiles for Biolistic Transformation of Plants.
P-1047 J. A. RUSSELL, J. L. Rasmussen, M. K. Roy, and J. C. Sanford.

10:45 A Monocot. Wound-Induced Promoter (AoPR1) is Specifically Expressed in Target Cells for Plant Transformation.
P-1048 S. FIREK, S. Özcan, S. Warner, and J. Draper.

11:00 A Simple Direct Technique of Transformation in Sunflower.
P-1049 A. ESPINASSE.

11:15 Negative and Negative-Positive Transformation Marker Combinations for Homologous Gene Replacement in *Arabidopsis* and Tobacco.
P-1050 L. MÁRTON and M. Czakó.

11:30 Optimization of Microprotoplast System for Limited Gene Transfer in Plants.
P-1051 K. S. RAMULU, H. A. Verhoeven, P. Dijkhuis, and J. Blaas.

11:45 Genetic Engineering of Multiple Virus Resistance in Plants: Data from Field Trials.
P-1052 H. D. Quemada, D. M. Tricoli, R. Z. Deng, P. F. Russell, J. R. McMaster, M. L. Boeshore, J. F. REYNOLDS, N. E. Bieber, D. W. Groff, K. Hadden, B. Moraghan, A. May, and J. P. Hubbard.

WEDNESDAY: 24 June **Regency A**

CONTRIBUTED PAPERS

V Neoplastic Cells and Carcinogenesis

Convener: Katherine Sanford, National Cancer Institute, USA

10:00 Fluorescent In Situ Suppression Hybridization for the Detection of the t(11;22)(q24;q12) in Primitive Neuroectodermal Tumors.
V-1025 M. GIOVANNINI, L. Selleri, M. Serra, M. A. Brunelli, and G. A. Evans.

10:15 Complementation of a DNA Repair Deficiency in Three of Four Human Tumor Cell Lines by Chromosome 4.
V-1026 K. K. SANFORD, F. M. Price, Y. Ning, and R. Parshad.

10:30 Oncogene Expression by a Mullerian Tumor Cell Line.
V-1027 J. L. BECKER, M. Finan, and R. H. Widen.

10:45 Alterations in the Steady-State mRNA Levels of c-Myc and p53 in L1210 Cell Lines Resistant to Deoxyadenosine.
V-1028 J. G. CORY, S. D. Long, C. E. Johnson, G. L. Carter, and A. H. Cory.

11:00 GST-P Expression and In Vitro Promotion of Neonatal Rat Hepatocytes Initiated In Utero with DMN.
V-1029 L. Testolin, L. Menapace, M. Ribecco, W. Jun, and U. ARMATO.

11:15 Antiproliferative Activity of Side Chain Derivatives of a Unique Antiestrogen (Analog II) on MCF-7 Human Breast Cancer Cells in Culture.
V-1030 L. M. OVERACRE, K. Avor, J. Kunchandy, and R. A. Magarian.

11:30 In Vitro Carcinogenesis of Mammary Epithelial Cells by N-Nitroso-N-Methylurea Using Collagen Gel Culture.
V-1031 J. R. LADUCA and D. K. Sinha.

11:45 Relationship Between DNA Topoisomerase I Gene Expression and Sensitivity to Camptothecin in Five Human Cancer Cell Lines.
V-1032 P. PEREGO, G. Capranico, G. Casati, and F. Zunino.

WEDNESDAY: 24 June **Regency B**

CONTRIBUTED PAPERS

V Studies of Differentiated Cells

Convener: Joji Ando, Tokyo University, Japan

10:00 The Long-Term Viability of Microvessel Endothelial Cells Derived from Human Liposuction Fat.
V-1033 C. B. HU, M. T. Ma, K. E. Myers, and R. C. Quijano.

10:15 Lymphatic Endothelial Cell Isolation, Culture and Characterization.
V-1034 L. V. LEAK.

10:30 Fibronectin-Mediated Elongation of Microvessels During Angiogenesis In Vitro.
V-1035 R. F. NICOSIA, E. Bonanno, and M. R. Smith.

10:45 Role of Basic Fibroblast Growth Factor in Rat Aortic Angiogenesis.
V-1036 S. VILLASCHI, R. F. Nicosia, and M. R. Smith.

11:00 Use of a Human Microvascular Endothelial Cell Line as a Model System to Evaluate Cholesterol Uptake.
V-1037 E. W. Ades and J. M. PRUCKLER.

11:15 Intracellular Calcium Response to Mechanical Shearing Force in Cultured Vascular Endothelial Cells.
V-1038 J. ANDO, A. Ohtsuka, R. Korenaga, and A. Kamiya.

11:30 Platelet-Derived Growth Factor (PDGF) Stimulates NaK-ATPase α_1 mRNA Levels in Cultured Rat Thoracic Aortic Smooth Muscle Cells.
V-1039 C. S. LO, T. Tamaroglio, and J. Zhang.

11:45 Long-Term Replicatively-Active Cell Cultures of Normal Adult Human Hepatocytes Express Liver-Specific Function.
V-1040 S. M. D'AMBROSIO and R. E. Gibson-D'Ambrosio.

12:00 Establishment and Long-Term Culture of Normal Human Kidney Glomerular Epithelial Cells.
V-1041 R. E. GIBSON-D'AMBROSIO and S. M. D'Ambrosio.

WEDNESDAY: 24 June **Potomac III**
14:00-17:00

LABORATORY MATERIALS AND BIOSAFETY COMMITTEE MEETING

WEDNESDAY: 24 June **Regency E-F**

SESSION-IN-DEPTH

P & I Identification and Expression of Insecticidal Proteins
Conveners: Frederick J. Perlak, Monsanto Company, USA and Bill McCarthy, Penn State University, USA

The recent interest in engineering plants for insect resistance has led to considerable research activity focused on the isolation and characterization of proteins in both hosts and their bacterial predators. This session will look at the molecular biology of these proteins and evaluate their potential use in transgenic crop plants as an alternative to the use of chemical insecticides.

14:00 The Commercialization of Insect Resistant Cotton: The Road from the Lab Bench to the Market-Place.
JS-9 F. J. PERLAK.
14:40 Searching for Insect Resistance Genes: Alternative Sources and Methods.
JS-10 L. L. MURDOCK.
15:20 Characterization of Insect Brush Border Proteins Specific to *Bacillus thuringiensis* Insecticidal Proteins by Toxin and Monoclonal Antibody Binding.
JS-11 M. ADANG, S. Paskewitz, and S. Garczynski.
16:00 Baculovirus-Mediated Expression of an Insect-Specific Scorpion Toxin.
JS-12 P. V. CHOUDARY, B. F. McCutchen, E. Fowler, B. D. Hammock, and S. Maeda.
16:30 Properties and Analysis of Insecticidal Bacterial Proteins Produced in Lepidopteran Cell Cultures Using Baculovirus Vectors.
JS-13 B. A. FEDERICI.
17:00 Discussion
B. McCARTHY.

WEDNESDAY: 24 June **Washington A-B**

SESSION-IN-DEPTH

P Molecular Genetics of Forest Trees
Conveners: Ron Sederoff, North Carolina State University, USA and Ralph Mott, North Carolina State University, USA

The constraints of environmentally sound management practice and long life cycles make forest species attractive candidates for genetic improvement by way of molecular genetics. Classical genetics has been hindered by the typically long life cycles. Recent progress in DNA mapping for both conifers and hardwoods is placing the needed new tools in our hands. Prior advances in tissue culture manipulation of tree species has been successfully coupled with advances in DNA manipulationof critical processes such as photosynthesis and lignin formation in wood. This session will show rapid progress by which forest species are advancing to assume a place along side agricultural species in molecular genetics, and exploring the unique biology of woody plants.

14:00 Gene Expression in Wood Formation and Genetic Mapping in Loblolly Pine.
P-22 R. SEDEROFF.
14:45 The Matching of Biological and Physical Parameters is Crucial for the Stable Genetic Transformation of Conifers.
P-23 D. D. ELLIS.
15:30 Genome Mapping in *Populus*: Genetic Dissection of Adventitious Shoot Formation In Vitro.
P-24 H. D. BRADSHAW, JR., K.-H. Han, B. D. Watson, and R. F. Stettler.
16:15 Structure and Evolution of Photosynthesis Genes in Pines.
P-25 P. GUSTAFSSON, J. Lidholm, S. Jansson, and A.-K. Lundberg.

WEDNESDAY: 24 June **Regency A-B**

SESSION-IN-DEPTH

Sponsored by the National Institute for the Advancement of In Vitro Sciences

T Characterization of Chemical Carcinogenesis In Vitro
Convener: Deborah D. Kaden, Health Effects Institute, USA

14:00 Introduction
14:10 In Vivo and In Vitro Comparisons of Mutational Changes.
T-30 B. W. GLICKMAN.
14:40 Malignant Transformation of Human Fibroblasts In Vitro.
T-31 J. J. McCORMICK and V. M. Maher.
15:10 Cytogenetic Approach to Document Factors that Contribute to the Development of Cancer.
T-32 W. W. AU.
15:40 Cultured Human Oral Epithelium: Growth, Transformation and Tobacco-Related Pathology.
T-33 R. GRAFSTRÖM, K. Sundqvist, P. Kulkarni, and Y. Liu.

WEDNESDAY: 24 June **Regency C-D**

SESSION-IN-DEPTH

V Cellular Aging
Conveners: Sydney Shall, University of Sussex, England and James R. Smith, Baylor College of Medicine, USA

14:00 TBA
V-30 H. ZUR HAUSEN.
14:30 Cell Cycle Regulation and Cellular Senescence.
V-31 J. C. BARRETT, C. A. Afshari, L. A. Annab, B. A. Burkhart, J. Boyd, R. D. Owen, P. A. Futreal, G. Preston, and K. H. Richter.
15:00 Transcription Factors, Proliferation and Cellular Aging in Human Fibroblasts.
V-32 J. CAMPISI.
15:30 Isolation of Genes for Cellular Mortalization.
V-33 S. SHALL, I. R. Kill, A. Broadhurst, and M. K. O'Farrell.
16:00 Role of Overexpressed Genes in the Replicative Senescence of Human Diploid Fibroblasts (HDF).
V-34 S. GOLDSTEIN.
16:30 Senescent Cell Derived Inhibitors of DNA Synthesis.
V-35 A. Noda, S. Venable, and J. R. SMITH.

WEDNESDAY: 24 June **Regency C**

BANQUET 20:00-22:00

THE CAPITOL STEPS

THURSDAY: 25 June **Potomac I-VI**

PLENARY SESSION

V Regulatory Issues of Biotechnology
Convener: Gretchen J. Darlington, Baylor College of Medicine, USA

8:00 Interests and Ethical Limits of Human Fetal Cells in Medical Research.
PS-11 M. ADOLPHE.
8:30 Biosafety, Research and Policy Challenges for Agricultural Biotechnology.
PS-12 A. L. YOUNG and M. K. Cordle.
9:00 Correspondence Between In Vivo and In Vitro Systems in Environmental Health Sciences.
PS-13 K. OLDEN.

THURSDAY: 25 June **Potomac V-VI**

SESSION-IN-DEPTH

T Culture of Neuronal Cells: Applications to Toxicology
Convener: T. K. Rowles, University of Tennessee, USA

10:00 Introduction
10:10 Designing an In Vitro Screening Battery to Evaluate Pesticide Neurotoxicity.
T-34 B. VERONESI.
10:45 The CytoFluor 2300 Fluorescent Plate Reader and a Multiple Endpoint Assay Reveals Neuroprotective Abilities of Nimodipine.
T-35 A. Danks, R. Isaacson, J. Im, and R. G. VAN BUSKIRK.
11:20 Effects of Lead on Ion Channels in Mouse N1E-115 Neuroblastoma Cells.
T-36 J. VAN DEN BERCKEN, H. P. M. Vijverberg, M. Oortgiesen, and T. Leinders.
11:55 In Vitro Evaluation of Neural Function.
T-37 T. K. ROWLES, D. Taylor, A. Nostrandt, and M. Ehrich.

THURSDAY: 25 June **Washington A-B**

SESSION-IN-DEPTH

V Endothelial Cell Cultures
Conveners: Thomas Maciag, American Red Cross, USA and K. Allen Brown, The Rayne Institute, England

10:00 Structure-Function Studies of FGF-1.
V-36 W. H. BURGESS.
10:45 Cellular Signals Controlling Endothelial Cell Proliferation and Gene Expression.
V-37 J. A. M. MAIER.
11:30 Gene Transfer in Human Endothelial Cells In Vitro.
V-38 V. GOPAL.
12:15 TBA
V-39 D. SHEPRO.

THURSDAY: 25 June **Regency F**

WORKSHOP

10:00 **Marine Plant and Animal Cell, Tissue and Organ Culture: Applications to Biotechnology**
Convener: TBA

THURSDAY: 25 June **Potomac III-IV**

WORKSHOP

10:00 **P Regulation of Plant Biotechnology**
Convener: J. Payne, USDA-APHIS, USA

This workshop will bring together regulators, technology users and research scientists to look at the impact of regulations on the use of biotechnology.

THURSDAY: 25 June **Potomac V-VI**

SESSION-IN-DEPTH

P Novel Plant Growth Regulators
Conveners: Ebrahim Firoozabady, DNA Plant Technology Corporation, USA and A. V. Roberts, Polytechnic of East London, England

In the past several years, novel plant growth regulators such as thidiazuron, CPPU, forchlorfenuron and DPU have been found to have very strong growth regulatory activities; much more than conventional growth regulators. These compounds have not been widely used in plant tissue culture, yet can produce results that are far superior to those observed using conventional protocols. This session will review the results on the use of novel growth regulators in the regneration of plants from recalcitrant and/or horticultural species.

14:00 Effectiveness of Thidiazuron and CPPU as Cytokinin-Like Compounds.
P-26 P. E. READ, G. Yang, and C. O. Auko.
14:30 Effects of Paclobutrazol on the Development In Vitro of Chrysanthemum, Rose and Grapevine.
P-27 A. V. ROBERTS, E. F. Smith, J. Mottley, and I. Gribaudo.
15:00 The Effects of Thidiazuron and Other Cytokinins on Plant Regeneration and Transformation in Carnation and Rose.
P-28 C.-Y. LU, G. Nugent, S. Tsuda, and R. Young.
15:30 TBA
P-29 K. TORRES.
16:00 Silverthiosulphate, Silvernitrate and Carbendazim as Alternative Plant Growth Regulators in Tissue Culture Systems.
P-30 P. C. DEBERGH, G. De Coster, A. Agneessens, and A. Jantasilp.
16:30 Growth Regulation and Hardening of Bioreactor Regenerated Plants by Growth Retardants.
P-31 M. ZIV.

THURSDAY: 25 June **Washington A-B**

SESSION-IN-DEPTH

P Control of Gene Expression in Plants
Convener: Dottie Pierce, Pioneer Hi-Bred International, Inc., USA

There are a variety of control mechanisms which do or can regulate the expression of genes in plants, whether those genes are endogenous or genetically engineered and introduced via transformation. In this session, we will examine the factors involved in regulating the expression of genes for several distinct plant systems at different times of development and in different tissues of the plant. Also to be discussed will be the use of mutant regulatory genes for modulating the expression of specific target genes.

14:00 Hierarchical Control and Hormonal Modulation of Gene Expression in Seed Development.
P-32 M. A. Bogue, J. Vivekananda, and T. L. THOMAS.
14:45 A Gene Network Controlling Glutamine and Asparagine Biosynthesis in Plants.
P-33 G. M. CORUZZI, F.-Y. Tsai, T. Brears, and G. Tjaden.
15:30 Functional Analysis of Pollen-Expressed Genes.
P-34 S. McCORMICK, J. Yamaguchi, L. Dircks, and K. Hamby.
16:15 Modulation of Maize Gene Expression Using Trans-Activators and Dominant Negative Inhibitors.
P-35 B. BOWEN, B. Drummond, E. Unger, L. Tagliani, S. Maddock, F. Solan, L. Sims, B. Roth, R. Parsons, R. Schmidt, E. Grotewold, and T. Peterson.

MONDAY: 22 June
8:00 a.m. to Midnight

TUESDAY: 23 June
8:00 a.m. to Midnight

WEDNESDAY: 24 June
8:00 a.m. to 15:00 p.m.

POSTER SESSION

Posters Mounted Sunday, 21 June, 21:00
Posters must be removed from Exhibit Hall
Wednesday, 24 June, 15:00

Authors Will be Present at Their Posters the Following Days and Times

Monday, 22 June		Tuesday, 23 June	
Odd Numbers	13:00-14:00	Even Numbers	13:00-14:00
Even Numbers	17:00-18:00	Odd Numbers	17:00-18:00

INVERTEBRATE CELL CULTURE

I-1015 Large-Scale Process Optimization for the Production of Recombinant Proteins in the Baculovirus Expression Vector System. G. P. GODWIN, W. G. Whitford, D. H. Calhoun, and S. A. Weiss.

I-1016 New Developments in BEVS Accessory Package Applications. W. G. WHITFORD, G. P. Godwin, and S. A. Weiss.

I-1017 A Simplified Serum-Free Powdered Insect Medium with Improved Performance Characteristics and Multiple Cell Line Applicability. V. CALDWELL, L. Tabor, and D. Kern.

I-1018 Insect Cell and Baculovirus Production in Serum-Free Media.B. W. BELISLE, C. Celeri, K. Tang, T. Montgomery, and T. Gong.

I-1019 Establishment of a Midgut Cell Culture from Lepidoptera. S. SADRUD-DIN, R. S. Hakim, and M. Loeb.

I-1020 Development of a Transposition Assay for Lepidopteran Transposons Using a Baculovirus Genome as a Target. M. J. FRASER, K. Boonvisudhi, K. Pecen, P. Brzezinski, T. Ciszczon, J. Pancheri, L. Cary, and H. Wang.

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PLANT TISSUE CULTURE

P-1053 Ethylene, CO_2 and O_2 Changes During Somatic Embryo Development in Liquid Cultures of *Ipomoea batatas*. M. E. BIENIEK, R. C. Harrell, and D. J. Cantliffe.

P-1054 Some Biochemical Aspects of Sodium Chloride Salinity Tolerance in Rice. V. A. CHAUHAN, P. C. Josekutty, L. Prakash, and G. Prathapasenan.

P-1055 Effects of Polyamines and Their Inhibitors on Organogenesis in *Euphorbia esula* L. D. G. DAVIS and P. A. Olson.

P-1056 Callus Culture and Regeneration of Sea Lettuce (*Ulva lactuca* L.). J. L. GALLAGHER and S. McElwee.

P-1057 Prolonged Storage of Grape Germplasm In Vitro. Influence of Low Temperature and Reduced Ammonium Nitrate Concentration. S. GANESHAN, R. Doreswamy, and D. G. Krishnappa.

P-1058 Field Evaluation of Herbicide-Tolerant Hybrid Poplar Somaclones. C. H. MICHLER, T. M. Voelker, and R. J. Moioffer.

P-1059 A Model for Study Disease Resistance in *Phaseolus vulgaris*. E. L. MYLES, R. Nesby, S. Bhatti, and C. Caudle.

P-1060 Tissue Culture Studies in Certain Mutants of Sunflower. T. PADMAVATHI, B. Pratibha Devi, and V. Kiranmai.

P-1061 Tissue Culture and Morphogenetic Studies in Clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.]. CH. AYODHYA RAMULU.

P-1062 Morphogenesis in Tissue Cultures of Various Explants of Cluster Bean [*Cyamopsis tetragonoloba* (L.) Taub.]. CH. AYODHYA RAMULU and D. Rao.

P-1063 Development of Embryogenic Tissue Culture and Subsequent Plant Regeneration from *Paspalum notatum* Fluegge "Tifton 9". R. A. Wheeler, R. G. SHATTERS, JR., and S. H. West.

P-1064 In Vitro Adventitious Root Production in *Artemesia annua* L. S. VENKETESWARAN, H. Nguyen, T. Doung, H. Samartzidou, and N. Kletzly.

P-1065 Somatic Embryogenesis in *Helianthus Porteri* (A. Gray) Heiser. W. F. YATES, JR.

P-1066 Tissue Culture and Regeneration in the Halophytic Grass *Spartina alterniflora* Loisel. X. LI, D. M. Seliskar, J. Moga, and J. L. Gallagher.

P-1067 Induction of Differentiation and Regeneration of Plants, Establishment of Fine Cell Suspension Cultures and Protoplast Isolation in Indica Rice Varieties. M. S. KHINDA, S. S. Gosal, J. S. Sandhu, and H. S. Dhaliwal.

P-1068 Somaclonal Variation—A Source of Genetic Diversity in Safflower, *Carthamus tinctorius* L. P. SEETA and S. Y. Anwar.

P-1069 Anther Culture of Indica Rice Varieties. J. S. SANDHU, M. S. Gill, S. S. Gosal, M. S. Khinda, and H. S. Dhaliwal.

P-1070 Somatic Embryogenesis and Plant Regeneration from Callus Cultures of Finger Millet. A. M. RAO.

P-1071 High Frequency of Plant Regeneration from Callus Cultures of *Paspalum scrobiculatum*. A. M. RAO.

P-1072 Somatic Embryogenesis in Hybrid *Liriodendron*. S. A. MERKLE, M. T. Hoey, B. A. Watson-Pauley, and S. E. Schlarbaum.

PLANT TISSUE CULTURE

P-1073 Mutliple Tillering Through Secondary Embryogenesis in Anther Culture of Indica Rice. N. LENKA and G. M. Reddy.

P-1074 Hormone-Mediated Regulation of Fatty Acids Production in Calli of *Euphorbia characias* L. M. FERNANDES-FERREIRA, J. M. Novais, M. Salomé, and S. Pais.

P-1075 Long-Term Preservation of Embryogenic Cell Cultures of Birch (*Betula pendula* Roth). L. M. MANNONEN and W. A. Monger.

P-1076 Selection of Glyphosate-Tolerant Tobacco Variants from Callus Cultures. M. NEDKOVSKA.

P-1077 Pollen Cultures of Pepper (*Capsicum annuum* L.). N. SIMEONOVA, R. Pandeva, and N. Zagorska.

P-1078 Optimization of Rice Protoplast Nurse Culture Using the Filter Method. J. R. AMBLER, J. W. Seay, and T. C. Hall.

P-1080 Carbohydrate Metabolism During Somatic Embryogenesis in Carrot. W. Y. SOH, Y. S. Kim, and D. Y. Cho.

P-1081 *Daucus carota*: Characterization of Cell Lines and Plants Resistant to Chlorsulfuron. S. Caretto, M. C. Giardina, C. Nicolodi, and D. MARIOTTI.

P-1083 Shoot Differentiation and Plant Regeneration of *Artocarpus heterophyllus* L. am. Through Callus Culture. S. K. ROY, T. Hossain, and M. S. Islam.

P-1084 The Effect of Sugars and Growth Regulators on Embryoid Formation and Plant Regeneration from Barley Anther Culture. Q. H. CAI and I. Szarejko.

P-1085 Isolation of High Yielding Line Through Tissue Culture in *Carthamus tinctorious* L. (Safflower). M. MADHAVA REDDY.

P-1087 Somatic Embryogenesis in *Vigna radiata* (L.) Wilczek. A. MADHU MOHAN RAO and S. A. Farooq.

P-1089 Tissue Culture and Biochemical Studies of Alternaria Resistance in Safflower (*Carthamus tinctorius* L.). M. A. KHADEER and S. Y. Anwar.

PLANT MICROPROPAGATION

P-1094 In Vitro Shoot Proliferation of *Astrophytum capricorne*. M. C. Ojeda, E. CARDENAS, T. E. Torres, and J. Verduzco.

P-1095 Evaluation of Somaclonal Variation in Ornamental Plants. S. MOHAN JAIN.

P-1096 Physiological Aspects of Vitrified Plants During Transformation to Normal Morphology. T. W. ZIMMERMAN and B. G. Cobb.

P-1097 Callus Induction and Regeneration in Leaf Blade Culture of Beta Species. P. AHMADIAN-TEHRANI and M. Mesbah.

P-1098 Establishment and Maintenance of Embryogenic Tissue Cultures of *Pinus strobus* L. K. KAUL.

P-1099 Micropropagation of Texas Madrone, *Arbutus texana*. W. A. MACKAY.

P-1100 Micropropagation of *Sequoia sempervirens*. I. W. SUL and S. S. Korban.

P-1101 Clonal Propagation of *Phoenix dactylifera* cv. Barhee Via Somatic Embryogenesis. S. Bhaskaran and R. H. SMITH.

P-1102 Rapid In Vitro Propagation of *Kielmeyera coreacea* Martius. J. E. PINTO and E. F. Arello.

P-1103 Factors Affecting In Vitro Rooting of *Quercus suber* L. A. ROMANO, C. Noronha, and M. A. Martins-Loução.

P-1104 Shoot Multiplication and Plant Regeneration from Shoot-Tip of *Gmelina arborea* by In Vitro Culture. S. K. ROY, J. Sen, and M. S. Islam.

P-1105 The Acclimatization of In Vitro Cultured Plantlets in the Breeding of Seedless Table Grapes (*Vitis vinifera*) in South Africa. I. A. TRAUTMANN and P. Burger.

P-1106 In Vitro Response of Excised Kentucky Bluegrass Seed. W. MSIKITA and H. T. Wilkinson.

P-1107 Effect of Growth Regulators on In Vitro Embryo Culture of *Carica papaya*. M. A. I. ALLOUFA.

P-1108 The Effect of Growth Retardants on Anthocyanin Production in Carrot Cell Suspension Cultures. A. ILAN and D. K. Dougall.

P-1109 Natural Products from Hairy Root Cultures of *Astragalus* Species. I. IONKOVA, Z. B. Hu, and A. W. Alfermann.

P-1110 Diterpenoids in Transformed Root Cultures of *Salvia miltiorrhiza*. Z. B. HU and A. W. Alfermann.

P-1111 Essential Oils in Different Calli of Garden Lovage. S. Y. ZHANG.

P-1112 A New Method for the Selection of Plant Pigment Producing Cells by Flow Sorting. K. SAKAMOTO, K. Iida, and T. Koyano.

PLANT SECONDARY PRODUCTS

P-1113 Obtaining of Highly Productive *Digitalis purpurea* Strains for Cardenolides in a Shoot Culture. S. KISE, Y. Hisatsugu, and Y. Yukimune.

P-1114 Analysis of Soluble Proteins During Somatic Embryogenesis in *Codonopsis lanceolata* L. D. Y. CHO, P. S. Choi, and W. Y. Soh.

PLANT TRANSFORMATION AND MOLECULAR BIOLOGY

P-1116 Transformation of Tomato (*Lycopersicon esculentum* L.) Medialed by *Agrobacterium tumefaciens*. H.-H. CHANG and K.-L. Chen.

P-1117 Production of Transgenic Rice Plants Using *Bar* Gene as a Selectable Marker. K. S. Rathore, V. K. CHOWDHURY, and T. K. Hodges.

P-1118 Transformation and Analysis of Two South African Tobacco Cultivars. S. J. Hearn, P. A. KOCH, and J. R. Webster.

P-1119 High Expression of a Foreign Gene in Transformed Bean Callus. C. I. FRANKLIN, T. N. Trieu, R. A. Dixon, and R. S. Nelson.

P-1120 Transformation of Another Solanaceous Species, *Datura innoxia Mill.* by Direct Gene Transfer and Regeneration of Transformed Plants from Protoplast Derived Calli. T. Schmidt-Rogge, M. MEIXNER, and O. Schieder.

P-1121 Development of Plant Regeneration and Transformation Systems in Sweet Potato (*Ipomoea batatas*). J. M. LOWE, C. A. Newell, F. Buitron, F. Medina, and J. Dodds.

P-1122 Expression of an Abscisic Acid Responsive Promoter in *Picea abies* (L.) Karst. Following Bombardment from an Electric Discharge Particle Accelerator. R. J. NEWTON, H. S. Yibrah, N. Dong, D. H. Clapham, and S. von Arnold.

P-1123 Genetic Transformation of Peanut Callus Via *Agrobacterium* Mediated DNA Transfer. C. I. Franklin, K. M. SHORROSH, B. G. Cassidy, and R. S. Nelson.

P-1124 Direct Gene Transfer Procedures for Eucalyptus Genetic Transformation. C. TEULIERES, N. Leborgne, and A. M. Boudet.

P-1125 Manipulation of Proline in Transgenic Cotton. N. L. TROLINDER and D. W. Ow.

P-1126 The Use of Microprojectile Bombardment in the Transformation of Peanut. J. A. SCHNALL and A. K. Weissinger.

P-1127 Development of a Haploid Sporophyte *Agrobacterium*-Mediated Transformation System in *Brassica napus*. M. ARNOLDO, N. MacLean, B. Huang, and R. Kemble.

P-1128 Micro-Electroporation: A Method of Introducing Macromolecules into Single Cells of Higher Plant Tissue. G. L. ZHU and T. L. Yan.

P-1129 Transformation and Analysis of Inducible PAL Genes in Potato. F.-F. LIN, J. Collier, L. Fitzmaurice, and E. L. Virts.

P-1130 Expression of *gus*A Gene with an Intron in Sweet Potato and Garden Egg Plant. A. POROBO-DESSAI, E. T. Blay, C. S. Prakash, and K. Nakamura.

P-1131 The Effects of Ultrasound on Uptake of Calcein and DNA by Protoplasts and Cells of Kentucky Bluegrass. H. Tang, G. Jelenkovic, and C. CHIN.

P-1132 Maize Haploid Protoplast Transformation and Regeneration of Transgenic Plants. K. SUKHAP-INDA, M. Kozuch, B. Rubin-Wilson, K. Smith, K. Crawford, W. Ainley, J. Mitchell, and D. Merlo.

P-1133 Transformation of *Lycopersicon esculentum* with an ACC Deaminase Gene to Inhibit Ethylene Production. S. VANDERPAN, V. Ursin, R. Sheehy, and W. Hiatt.

P-1134 Transformation of an Elite Maize Inbred Through Microprojectile Bombardment of Regenerable, Embryogenic Callus. K. AVES, D. Genovesi, N. Willetts, S. Zachwieja, M. Mann, T. Spencer, C. Flick, and W. Gordon-Kamm.

P-1135 Isolation of Immature Inflorescence and Ear Protoplasts and Their Culture in Cereals. X. Y. CHENG.

P-1136 Development of Glyphosate as a Selectable Marker for the Production of Fertile Transgenic Corn Plants. A. HOWE, F. Tamayo, S. Brown, C. Armstrong, M. Fromm, J. Hart, S. Padgette, G. Parker, and R. Horsch.

P-1137 Expression of an ABA Responsive Promoter in *Pinus elliottii* Following Gas-Driven Particle Bombardment. N. DONG and R. J. Newton.

P-1138 Biochemical, Histological, and Molecular Characterization of Haustorial Development in Root Cultures of *Striga asiatica*. S. J. WOLF and M. P. Timko.

PLANT TRANSFORMATION AND MOLECULAR BIOLOGY

P-1139 Analysis of DNA Polymorphism by Random Amplified Polymorphic DNA Markers in Anther Culture Derived Broccoli Plants. F.-S. Wu and C.-L. LAN.

P-1140 Transient Gene Expression in Electroporated Medicago Protoplasts. G. P. KOSTURKOVA and M. R. Davey.

P-1141 Expression of a Cyclophilin:GUS Chimeric Gene in Transgenic Rice. W. G. BUCHHOLZ, J. W. Seay, R. T. DeRose, and T. C. Hall.

P-1142 Isolation of Triosephosphate Isomerase Gene from Rice. Y. XU and T. C. Hall.

P-1143 Stable Transformation of Sweet Potato by Electroporation. M. NISHIGUCHI, Y. Uehara, and K. Komaki.

P-1143A Field Evaluation of Herbicide-Tolerant Hybrid Poplar Somaclones. C. H. MICHLER, T. M. Voelker, and R. J. Moioffer. (See Abstract P-1164).

P-1143B Expression of Rol Genes and Insurgence of Genetic Tumors in *Nicotiana glauca* x *Nicotiana langsdorfii* Hybrids. D. MARIOTTI, M. Cardarelli, P. Bogani, M. Buiatti, and P. Costantino. (See Abstract P-1160).

PLANT MORPHOGENESIS

P-1144 Regeneration of Kenaf, *Hibiscus cannabinus* L. Plants from Explants and Tissue Cultures. H. SAMARTZIDOU, S. Venketeswaran, N. Kletzly, and H. Nguyen.

P-1145 Protein Synthesis During Carrot Embryogenesis and Germination: Comparison of Zygotic, Somatic and DFMA-Treated Somatic Embryos. R. P. FEIRER and P. W. Simon.

P-1146 Somatic Embryogenesis and Plant Regeneration in Zoysiagrasses (*Zoysia* spp.). H. Y. Noh, G. H. Park, J. S. Choi, and B. J. AHN.

P-1147 Somatic Embryogenesis of High Latitude-Adapted Soybean Cultivars and Germplasms. L. TIAN, D. C. W. Brown, and J. Webb.

P-1148 Induction of Desiccation Tolerance in Cultured Immature Embryos of Wheat. D. C. W. BROWN, C. J. Ledderhof, and J. A. Simmonds.

P-1149 Screening of *Medicago trautvettezi* and *Medicago falcata* Germplasm for Somatic Embryogenesis. E. SVANBAEV, I. Zadorozhnaya, E. Dzhangalina, and D. C. W. Brown.

P-1150 Involvement of Glutamate Dehydrogenase in Induction of Alfalfa Somatic Embryogenesis. K. I. FINSTAD, D. C. W. Brown, and K. W. Joy.

P-1151 Induction of Callusogenesis and Organogenesis in Mature Seeds of *Triticum aestivum* L. N. A. ZAGORSKA and V. Ilcheva.

P-1152 Two Novel Auxin Substitutes for Plant Tissue Culture. J. PONSAMUEL and P. Dayanandan.

P-1153 Some Bioechemical Changes During Callusing and Differentiation of Tree Species In Vitro. P. K. Chand and M. DWARI.

P-1154 In Vitro Morphogenesis from Cotyledon Derived Nodular Callus of Pigeon Pea. A. SAGAR and K. Vaidyanath.

P-1155 Somatic Embryogenesis and Organogenesis in the Himalayan Yellow Poppy *Meconopsis paniculata*. I. M. SULAIMAN, N. S. Rangaswamy, and C. R. Babu.

P-1156 A Two-Dimensional Electrophoretic Analysis of Protein During Somatic Embryo Formation in Cell Suspension Cultures of *Oryza*. K. OZAWA, D. H. Ling, and A. Komamine.

P-1157 Embryogenesis Specific Protein Changes in Birch (*Betula pendula* Roth.) In Vitro Cultures. A. K. HVOSLEF-EIDE and F. M. K. Corke.

P-1158 First Enzyme of the Shikimate Pathway Enhancement with the Differentiation of Xylem in Tobacco Callus. J. E. PINTO, J.-M. Zhao, and K. M. Herrmann.

P-1160 See "Plant Transformation and Molecular Biology" #P-1143B.

P-1163 Effect of Culture Filtrate of *Fusarium udum* on In Vitro Induced Embryos from Cotyledols of *C. cajan* Genotypes. D. M. BARVE, D. B. Patel, N. Badri, and A. R. Mehta.

P-1164 See "Plant Transformation and Molecular Biology" #P-1143A.

MONDAY: 22 June
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TOXICITY MODEL SYSTEMS

T-1016 A Cell Culture Model for Lung Toxicity. K. A. COSTA and J. M. Cerreta.

T-1017 An Automated Kinetic Microassay for Lactate Dehydrogenase Using a Microplate Reader. P. McNEELA and P. Dehn.

T-1018 Alterations in Lectin Binding to the Plasma Membrane of Cultured Epithelial Tracheal Cells After Incubation in Acidic Buffer. M. L. BERTHEZENE, F. Rogerieux, M.-A. Nahori, J. Bignon, and C. Lambré.

T-1019 Testosterone-Induced Ultrastructural Changes in Primary Neonatal Rat Myocardial Cell Cultures. R. MELCHERT, T. Herron, and A. Welder.

T-1020 Induction of Tumor Necrosis Factor Activity in Laboratory Animals Treated with Hepatotoxicants. A. ROY, G. R. Soni, R. M. Kolhapure, K. Banerjee, and P. S. Patki.

TOXICITY OF CHEMICALS AND DRUGS

T-1021 A Novel Analytical System to Monitor Cell Behavior in Response to Chemical Agents. C. R. KEESE and I. Giaever.

T-1022 Cytotoxicity, DNA-Binding and Immunofluorescent Localization of 3'-azido-2',3'-dideoxythymidine (AZT) in Human, Hamster and Mouse Cell Lines. O. A. OLIVERO, F. A. Beland, and M. C. Poirier.

T-1023 An In Vitro Screening System for the Nephrotoxicity of New Platinum Coordination Complexes Using Rabbit Kidney Proximal Tubule Cells in Primary Cultures. H. TOUTAIN, F. Courjault, D. Leroy, I. Coquery, and P. Mailliet.

T-1024 Potential Anti-Leukemic Activity of Monomethyl Glycol Ethers Evaluated by In Vitro Inhibition of Human (HL60) and Mouse (L1210) Leukemia Cell Culture Growth. J. A. GARNETT and M. P. Dieter.

T-1025 Risk Assessment of HIV/Infectious Disease in the Clinical and Research Laboratory: An Update. S. L. SCHNEIDER and P. Lincoln Smith.

T-1026 In Vitro Neurotoxicity of Anti-AIDS Nucleoside Analogs. A. E. Weiss, M. J. PALMOSKI, O. P. Flint, and F. B. Oleson.

T-1027 Vinblastine and Colchicine Cause Rapid and Pronounced Inhibition of Fast Axonal Transport Preceeding Neurite Degeneration in N1E.115 Neuroblastoma. D. J. BRAT and W. S. Brimijoin.

T-1028 Gap Junctional Intercellular Communication and Screening of Potential Tumor Promoters. E. HONIKMAN-LEBAN and M. M. Shahin.

T-1029 Evaluation of the Compounds Patulin, Penicillium Roqueforti Toxin, Botryodiplodin, and Palytoxin in the Metabolic Cooperation Assay. E. HONIKMAN-LEBAN and M. M. Shahin.

T-1030 In Vitro Toxicological Evaluation of Zidovudine (ZDV), Itraconazole (IC) and Rifabutin (RIF) Alone and in Combination. B. C. FOSTER, M. A. Bayne, D. L. Wilson, and S. R. Khan.

IN VITRO TOXICITY ASSAYS

T-1031 Testing Contact Lens Solutions on Serially Cultured Corneal Cells. P. A. Simmons, J. O. LaMotte, and C. Orenic.

T-1032 The Trans-Epithelial Permeability Assay as an In Vitro Assay for Predicting Ocular Irritation of Surfactant Formulations. K. M. MARTIN and C. W. Stott.

T-1033 Agar Diffusion Cytolysis: An Alternative Screen for the Prediction of a Corrosive Ocular Response. D. A. LASKA, J. T. Reboulet, J. O. Houchins, and R. L. St. Clair.

IN VITRO TOXICITY ASSAYS

T-1034 Comparison of the Responses of Human Skin to the Responses of the Living Skin Equivalent to Two Classes of Surfactant. R. GAY, M. Swiderek, A. Ernesti, and A. M. Kligman.

T-1035 Comparison of Several Methods Employed in In Vitro Cytotoxicology/Growth Assays. T. K. JOHNSON.

T-1035A Evaluation of a Group of Petrochemicals Using Clonetcs' Neutral Red Bioassay to Predict Irritancy. R. BARSTAD, J. Janus, J. Lauten, N. Accomando, A. Triana. (See Abstract V-1077).

ENVIRONMENTAL TOXINS

T-1036 Arsenic Induced Chromosome Changes in Cultured CHO Cells. W. Howard, S. Hoffman, and T. S. KOCHHAR.

T-1037 In Vitro Effects of Heavy Metals on the Nonspecific Defense Mechanisms and Immune Response of Fish. G. JENEY, D. Anderson, and Z. Jeney.

T-1038 Development of a Cell Line of Hepatocytes from Atlantic Menhaden (*Brevoortria tyrannus*). M. FAISAL, S. Sami, B. J. Rutan, D. E. Holmes, and S. F. Hoegerman.

T-1039 Macromolecular Synthesis in Cultured Hepatocytes of Spot (*Leistomus xanthurus*) from Polycyclic Aromatic Hydrocarbon Contaminated Environment. N. J. MORSE, M. Faisal, and R. J. Huggett.

T-1040 In Vitro Culture, Differentiation, and Macromolecular Synthesis of Eye Lens Epithelium of Cataractous and Non-Cataractous Spot (*Leiostomus xanthurus*). C. D. WILLIAMS, M. Faisal, and R. J. Huggett.

T-1041 Chromium Toxicity in *N. crassa*. V. VENKATA RAMANA.

T-1042 Pyrazolines and their Derivatives are Known to Possess Antibacterial, Antifungal and Antiviral Activity. B. SEENAIAH and D. Bhaskhar Reddy.

T-1043 A Quantitative In Vitro Test for the Detection of Waterborne Toxins. J. J. ALEXANDER and E. M. Bey.

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DIFFERENTIATED CELL TYPES

V-1042 Coculture of Normal Human Small Intestine Cells in a Rotating-Wall Vessel Culture System. T. J. GOODWIN, W. F. Schroeder, D. A. Wolf, and M. P. Moyer.

V-1043 A Model of Neural Cell Wounding and Repair: Electrophysiological and Morphological Indices. W. GROSVENOR, A. A. Messier, and H. W. Fisher.

V-1044 Polyamine-Phospholipide Complex (CDM) Bloks NMDA Activated Currents and Protects Neurons to Traumatic Injuries. A. T. GYEVAI and V. Janossy.

V-1045 Isolation of Putative Mesenchymal Stem Cells from Rat Embryonic and Adult Skeletal Muscle. P. A. LUCAS, A. F. Calcutt, D. J. Mulvaney, H. E. Young, and S. S. Southerland.

V-1046 Growth Factor Effects and Myo-D Expression in Cultured Balb/c and Swiss Mouse Muscle Cells. M. A. L. MALEY, Y. G. Fan, M. D. Grounds, and J. Rossi.

V-1047 Isolation of Putative Mesenchymal Stem Cells from Embryonic Chick Heart. M. L. MANCINI, R. P. Wright, P. A. Lucas, and H. E. Young.

V-1048 Regulation of AFP Synthesis in the OR-HEPA Cell Line In Vitro and In Vivo. M. H. MORALES, N. Cordero, and M. Urdaneta.

V-1049 Hepatocyte (HC) Cell Density Influences the Induction of Nitric Oxide (N=O) In Vitro. M. Di Silvio, D. Geller, V. Chough, T. R. Billiar, Z. Liu, R. L. Simmons, and A. K. NUSSLER.

V-1050 Confocal Imaging of Cultured Human Hepatocytes Infected with *Plasmodium falciparum* in the SCID Mouse Model. J. B. Sacci, Jr., L. Kubiak, A. Azad, J. Cottrell, K. Saccamato, R. Ascione, T. Papas, and J. RESAU.

V-1051 An Electron Microscopic Study of Chick Iris Melanocytes In Vitro. L. SARTORI.

V-1052 Testosterone Metabolism in an In Vitro Skin Model. S. R. SLIVKA, L. Landeen, and R. L. Bartel.

V-1053 Cultured Keratinocyte Allografts Accelerate Healing of Split Thickness Donor Wounds. R. Fratianne, I. Housini, and I. A. SCHAFER.

V-1054 Isolation of Putative Mesenchymal Stem Cells from Embryonic Chick Skin. R. P. WRIGHT, M. L. Mancini, P. A. Lucas, and H. E. Young.

V-1055 A Molecular Analysis of Mouse Nephrogenesis in an Organ Culture System. H. YEGER, D. Forget, A. Flenniken, C. Campbell, and B. R. G. Williams.

V-1056 Isolation of Putative Mesenchymal Stem Cells from the Skeletal Muscle of Embryonic Chick and Postnatal Mouse. H. E. YOUNG and P. A. Lucas.

V-1057 A Possible Role for Lactate in the Control of Differentiation in Cells Secreting Protein Products. C. MOTHERSILL, S. O'REILLY, M. Lehane, and C. Seymour.

V-1058 Human Biliary Epithelial Cells for In Vitro Studies of Biliary Atresia and Other Cholangiopathies. M. A. MORSE, S. T. Boyce, C. C. Daugherty, and M. M. Ziegler.

V-1059 Characterization of MDBK Cell Response to Epidermal Growth Factor: Mitogenesis and Receptor Binding. W. X. BALCAVAGE, A. Reilly, and R. Moore.

V-1060 Factors Influencing Granule Cell Collateral Sprouting in Organotypic Explants of Mouse Hippocampus. B. W. COLTMAN, E. M. Earley, and C. F. Ide.

V-1061 Intranuclear Actin and snRNP Aggregates Localize to Discrete, Mutually Exclusive Loci in Cultured DRG Neurons and Associated Non-Neuronal Cells. D. J. SAHLAS, K. Milankov, P. C. Park, and U. DeBoni.

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V-1062 Characterization of Enhanced Transglutaminase Catalysis in WI-38 Human Lung Fibroblasts by Ionophore A23187. M. D. MAXWELL, M. K. Patterson, Jr., P. J. Birckbichler, T. R. Norton, B. M. Fraij, G. L. Thomas, and H. A. Carter.

V-1063 Localization of Cellular Transglutaminase on the Extracellular Matrix After Wounding. H. F. UPCHURCH, E. Conway, M. K. Patterson, Jr., and M. D. Maxwell.

V-1064 Involvement of C/EBP Family Proteins and NF-*k*B-Like Factors in Regulation of Complement C3 Gene Expression. T. S. JUAN, D. R. Wilson, and G. J. Darlington.

V-1065 Steroid-Responsive Rat Primary Myometrial Cell Cultures for the Study of Gap Junctions. M. S. MARTY and R. Loch-Caruso.

V-1066 Keratinocytes Stimulate Murine Megakaryocytopoiesis. G. D. KALMAZ, E. E. Kalmaz, and M. M. Guest.

V-1067 Induction of TNFα Activity in Mice Experimentally Infected with Japanese Encephalitis Virus. G. R. Soni, K. Banerjee, R. M. Kolhapure, C. N. Dandawate, and A. ROY.

V-1068 Effect of In Vitro Infection with Ureaplasma Diversum on Endometrial Cell Function. J. J. Kim, P. A. Quinn, and M. A. FORTIER.

V-1068A The Adaptation of Rabies Virus in Vero Cell and in Human Diploid Cell Lines. W. TAI-CAI. (See Abstract V-1100).

V-1068B The Cell Line Data Base Gives Detailed Information on Human and Animal Cell Lines. B. PARODI, A. Manniello, O. Aresu, P. Romano, B. Iannotta, G. Rondanina, and T. Ruzzon.

IN VITRO BIOTECHNOLOGY

V-1069 Identification of a Novel Low Molecular Weight Growth-Promoting Factor Derived from Human Leukemia Cell Lines. T. DAO, S. Nakamura, V. Holan, and J. Minowada.

V-1070 Growth and Production of Hybridoma in a Lipid Containing Serum-Free Medium. K. HANSEN.

V-1071 Efficient Production of Recombinant α-Amidating Enzyme in a Stirred Tank Bioreactor. D. JACKSON-MATTHEWS, C. Pray, and K. Piparo.

V-1072 Gram Quantity Monoclonal Antibody Production in Continuous Bench-Scale Bioreactor Culture. W. MAGARGAL and A. Abramovitz.

V-1073 Three-Dimensional Modeling of T-24 Human Bladder Carcinoma Cell Line: A New Simulated Microgravity Vessel. T. L. PREWETT, T. J. Goodwin, R. P. Schwarz, and G. F. Spaulding.

V-1074 Prescreening of Potential Chemopreventive Agents Using Biomarkers of Transformation. S. SHEELA, J. D. Stutzman, E. Korytynski, K. R. Garris, and V. E. Steele.

V-1075 Use of Expanded Surface Roller Bottles for Production of Marek's Disease Virus Vaccine. J. R. MOLDENHAUER, T. H. Robinson, L. Ryan, and B. Nordgren.

V-1076 In Vitro Studies of Endogenous Adenine Nucleotide Contents in Relation to Sperm Motility and Acrosomal Protease Activity. J. S. CHEN, M. G. Menesini Chen, C. Sensini, M. Bari, M. Barbetti, and B. Baccetti.

V-1077 Has Been Moved to T-1035A

V-1078 Update on the Incidence of Mycoplasma Contamination Detected in Cell Lines and Their Products. J. LUCZAK, S. A. Knower, M. S. Cox, J. Dubose, Jr., and J. W. Harbell.

V-1079 Major Metabolic Changes Accompany Transfection and Selection for High Level Expression of Recombinant Genes. S. GOULD, D. DiStefano, G. Cuca, D. Robinson, and M. Silberklang.

V-1080 Correlation of Bacteriophage and Endotoxin Results in Animal Serum Products. J. D. KEATHLEY.

V-1081 The Effect of Serum Filtration on Cellular Attachment and Growth. M. MAZUR-MELNYK and A. L. Symington.

V-1082 Cell Culture Optimization and Cell Bank Establishment of the Human Cutaneous T Cell Lymphoma Cell Line HuT 78. M. MAZUR-MELNYK and M. Lacatus.

V-1083 Large-Scale Serial Subcultivation of MRC-5 Cells on Microcarriers. M. MAZUR-MELNYK, R. Warren, M. Krapez, H. Cho, and D. Alkema.

V-1084 Towards an Improved Control of the Environment for Animal Cells. J. M. Coco Martin, D. E. Martens, C. A. M. van der Velden-de Groot, R. C. Dorresteijn, B. Romein, and E. C. BEUVERY.

V-1085 Optimal Cryopreservation of a Human Skin Equivalent Can be Determined with the Multiple Endpoint Assay. L. S. RHOADS, J. G. Baust, and R. G. Van Buskirk.

V-1086 Plating Embryonic Chick Mesenchymal Cells in Serum-Free, Defined Media. S. S. SOUTHERLAND, H. E. Young, and P. A. Lucas.

IN VITRO BIOTECHNOLOGY

V-1087 Development and Characterization of a Cell Line Derived from a Diploid Frog, *Xenopus laevis*, Homozygous for all Loci. E. M. EARLEY, D. C. Reinschmidt, and R. Tompkins.

V-1088 DNA Fingerprinting: A Powerful Tool for the Differentiation of Cell Lines. G. N. STACEY, B. J. Bolton, and A. Doyle.

V-1089 Development of Procedures for the Optimization of Electrofusion Protocols. R. HELLER, M. Jaroszeski, and R. Gilbert.

V-1090 Fusion from Within: Light Microscopic and SEM Observations of LLC-RK1 Cell Monolayers Infected with Equine Herpesviruses. E. E. BRAKO, L. Phaire-Washington, and L. Brako.

V-1091 Cells on Rotating Fibers for Fast Reactions. R. A. CLYDE.

V-1091A Animal Cell Culture Collection in Russia. I. I. FREEDLASKAVA, G. G. Polanskaya, T. N. Efremava, and G. P. Pinaev. (See Abstract V-1105).

TUMORIGENESIS AND CHEMOPREVENTION

V-1092 Lead Ammonium Citrate Acetate (LACA) Prevents Spontaneous Apoptosis of Primary Adult Rat Hepatocytes in Synthetic Medium. M. Ribecco, E. Tognana, L. Testolin, L. Menapace, and U. ARMATO.

V-1093 Species Specificity of In Vitro Transformation of Tracheal Cells by N-methyl-N'-nitro-N-nitrosoguanidine. M. J. W. CHANG and C. C. Tsai.

V-1094 A Microtiter Enzyme Linked Immunosorbent Assay for Protein Tyrosine Phosphatase. S. MISHRA and A. W. Hamburger.

V-1095 Correlation Between In Vitro and In Vivo Induced Carcinogenicity by DDT. P. Balakrishnamurthy and K. REVATHI.

V-1096 Screening of Chemopreventive Agents Using the A427 Human Tumor Cell Anchorage-Independence Assay. E. A. KORYTYNSKI, S. Sheela, and V. E. Steele.

V-1097 In Vitro Prescreening of Chemopreventive Agents Using Assay for GSH and Free Radical Formation. J. D. STUTZMAN, K. Garris, and S. Sheela.

V-1098 Biological Activity and Chemical Class of 125 Chemopreventive Agents in Relation to Efficacy in the Rat Tracheal Epithelial Cell Foci Inhibition Assay. B. P. WILKINSON, J. T. Arnold, S. Sheela, and V. E. Steele.

V-1099 Evaluation of Potential Chemopreventive Agents Using Inhibition of Carcinogen-Induced Calcium Tolerance Assay. G. P. WYATT, E. L. Elmore, S. Sheela, and V. E. Steele.

V-1100 See "Differentiated Cell Types" #V-1068A.

V-1101 A Murine Myelomonocytic Leukemia Cell Line Resistant to Ciprofloxacin. A. PESSINA, E. Mineo, L. Gribaldo, and M. G. Neri.

V-1102 Growth Characteristic and Cytogenetic Analysis of Cellular Clones Derived from LoVo Cells with Intrinsic Different Sensitivity to Doxorubicine. T. Dasdia, M. Romagnoni, E. Scanziani, C. Barbieri, and E. DOLFINI.

V-1103 See "Differentiated Cell Types" #V-1068B.

V-1104 Read by Title.

V-1105 See "In Vitro Biotechnology" #V-1091A.

V-1106 Fish Cell Culture as a Mean in Studying Fish Reproduction. J. Galas, P. Epler, and S. STOKLOSOWA.

V-1107 Do Sertoli Cells Co-Cultured with Leydig Cells Influence the Arrangement of Their Cytoskeleton? B. BILINSKA.

V-1108 Histamine-Induced Contrasuppression in Bone Marrow. N. BELYAEV, G. Zakiryanova, and A. Beklemishev.

PS-1 **Micro-targeting Allows for Precise and Routine Acceleration of Foreign Genes into Shoot Meristems of Cereals.**
I. POTRYKUS, R. Bilang, V. Iglesias, A. Gisel, S. Zhang, D. Stein, N. Leduc and C. Sautter. Institute of Plant Sciences, Swiss Federal Institute of Technology (ETH) 8092 Zürich, Switzerland

Genetic transformation of cereals is still a major problem. Although *Agrobacterium tumefaciens* has been shown to transfer T-DNA also into cereal cells, transgenic cereals have not been recovered despite a great variety of experimental approaches. Direct gene transfer to protoplasts has yielded transgenic rice and maize but is, with the exception of Japonica rice varieties, far from being routine. Biolistic treatments of embryogenic suspension cultures have produced, in part after an enormous input in manpower, transgenic maize, and from scutellum treatments, transgenic rice. Again there is considerable room for improvement.

The key problem in cereal transformation is probably not delivery of DNA into cells, but delivery into totipotent cells which are obviously very rare in a cereal plant. There is, however, a small group of cells for which it is well established that it will form a plant: the shoot meristem. The true shoot meristem is a tiny structure of ca. 100 x 50 µm consisting of ca. 50 - 100 cells. Routine transformation of the cells of cereal shoot meristems *in vitro* could be expected to constitute a considerable advancement of transformation technology. Unfortunately the short meristem appears not to respond favourably to attempts to transformation by *Agrobacterium* nor to micro-injection and it is obviously difficult to hit it by the traditional biolistic devices.

We have developed an instrument (C. Sautter et al., Bio/Technology *9*: 1080-1085, 1991) which fullfills all technical requirements for reproducible, routine and precise acceleration of DNA-carrying individual micro-particles into (statistically) every cell of a cereal shoot meristem. We have shown that the genes are active in these meristematic cells. We have targeted shoot meristems of wheat, maize, barley, rice, millet, sorghum and other gramminaceous species. The shoot meristems regenerate efficiently to mature plants and we will study whether or not this gene targeting to the cells of cereal shoot meristems will lead to integrative transformation and transgenic offspring. We are well aware that the presence of foreign genes in a "meristematic" cell does not guarantee integrative transformation.

PS-2

Cytokine Stimulation and Cell Interactions Affect Retroviral Vector-Mediated Gene Transfer into Mouse and Human Hematopoietic Stem Cells. J.W. BELMONT, K. Moore, and F.Fletcher. Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas, 77030.

Introduction of novel genetic elements into hematopoietic stem cells (HSC) is one possible approach to the delivery of therapeutic agents in vivo. We evaluated conditions for stimulation of murine HSC transduction by retrovirus vectors. Interleukins 3, 6 and 7, Granulocyte Colony Stimulating Factor (G-CSF), and Leukemia Inhibitory Factor (LIF) were tested for effects on gene transfer efficiency in long term bioassays. Using a novel competitive repopulation assay we have shown that IL-3 and LIF but not IL-6 or G-CSF greatly enhance the repopulation potential of bone marrow stem cells after short term culture. Secondary CFU-S assays showed that the vector transduction efficiency into stem cells was approximately 70%. All bone marrow transplant recipient animals had expression of the transferred gene (human adenosine deaminase) in all of their hematopoietic tissues 6 - 10 months post transplant at levels which were near or exceeded the endogenous enzyme.

We also examined methods for transduction of primitive human hematopoietic progenitors - long term culture initiating cells (LTCIC). Competitive long term culture assays were established by differential marking of control and experimental cell populations with two vectors distinguishable by PCR. These assays were used to test the effects of growth factor stimulation (IL-3, IL-6, MGF, and LIF) and the effect of stromal cell monolayers on human stem cell transduction. Stromal support allowed 65% transduction efficiency that was not supplanted by exogenous growth factors. These data raise a number of questions about the control of stem cell growth in culture and suggest promising protocols for clinical experiments.

PS-3 Demetrios Spandidos
National Hellenic Research Foundation, Greece

Electroporation as Compared to Conventional Gene Transfer Techniques in the Study of Gene Function

NO ABSTRACT SUBMITTED

PS-5

An Efficient Method for Isolating and Mapping Human Genes.
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Gene identification and mapping efforts in humans have made it possible to map 3-4% of the human genome during the past 25 years. Newer methods promise to accelerate the pace of gene discovery and mapping. We have developed an integrated approach which permits identification of genes encoded by large fragments of human DNA cloned into yeast artificial chromosome (YAC) vectors and mapping them to specific locations on the YAC's. Our procedure involves construction of a highly comprehensive human cDNA library. This library, which contains at least 75-80% of all genes encoded by the human genome, is prepared by use of random primers and the polymerase chain reaction. cDNA's corresponding to genes located on specific YAC's are isolated by a DNA hybridization method. The affinity purified cDNA's are then mobilized into a yeast chromosome fragmentation vector and the minilibrary is used to target the corresponding exons by homologous recombination. This method permits gene isolation, understanding of intron-exon organization of individual genes, and allows easy access to the regulatory regions of genes. Genes encoded by specific regions of human chromosomes can be readily identified by this method.

PS-6

Transposable elements as tools to isolate and characterize genes from Antirrhinum majus

Three transposable element systems from Zea mays and several from Antirrhinum majus have proven useful in the isolation and characterization of genes involved in anthocyanine or starch biosynthetic pathways or in developmental processes. The strategy of transposon tagging of genes involved in flower development in Antirrhinum majus will be described as well as their isolation using differential cDNA cloning. The allocation of cloned cDNA's to known genetical loci makes use of the unstable nature of transposable element induced mutations.

PS-8

Global and Local Regulation of Pattern Formation during Insect Development. S.B. CARROLL, Howard Hughes Medical Institute and Laboratory of Molecular Biology, University of Wisconsin - Madison, Madison, WI 53706

During animal development, cells of the growing embryo become organized into structures of widely varying number, size, shape, composition, and function. Genetic and embryological studies of a model animal, the insect *Drosophila melanogaster*, have shown that the specification of the overall body plan, the formation of complex arrays of tissues and organs, and the assignment of individual cell fates occurs in a highly regulated temporal sequence and spatial pattern. Genetic screens for pattern-disrupting mutations have identified many of the loci which influence the size, shape, number, and/or function of body structures and have catalyzed an integrated approach to understanding the control and function of pattern-regulating genes and the overall logic of developmental processes.

Our research is currently focused upon the genetic control of pattern formation in insects at two levels. The first concerns the formation of large-scale patterns and the regulation and function of several globally-acting genes that establish segment number and segmental pattern in the larva and one gene that controls wing formation in the adult. The second level concerns pattern formation on the cellular level within the developing larval and adult nervous systems and focuses on the regulation of the so-called "proneural" genes that are required for neurogenesis.

PS-7

Cloning of a New Genes Concerning Cell Proliferation. T.Marunouchi and H.Hosoya. Division of Cell Biology. Institute for Comprehensive Medical Science, Fujita Health University, Toyoake Aichi 470-11, Japan

A human monocytic cell line, U937 can be differentiated into mature macrophages following induction with 12-o-tetradecanoyl-phorbol-13-acetate (TPA). The differentiated macrophages de-differentiate to proliferating U937 cells after prolonged culture with frequent medium changes. Using this culture system we can study either genes activated by differentiating agents and suppressed by de-differentiation or genes suppressed at differentiation and re-activated at de-differentiation. Pr22 is one of the genes thus cloned from a cDNA library by the differential hybridization method and actively transcribed in proliferating cells while completely suppressed at differentiated state. It is 1640bp long and contains an open reading frame of 447bp. The chromosomal Pr22 gene is about 12kbp long and constituted of at least 6 exons and 5 introns. According to the putative amino acid sequence, it contains a sequence of PLSPPKKK which is a potential substrate of $p34^{cdc2}$ kinase and at the same time similar to nuclear localization signal. Pr22 is transcribed in late S and G_2 phase in the cell cycle. To analyze the function of the product of this gene, a 5' terminal cDNA fragment was integrated into a plasmid containing metalothionein promoter in the reverse direction and transfected into U937 cells. By inducing antisense RNA synthesis, the growth of transformants was repressed. Cytofluorometrical analysis revealed that cells were arrested at G_2 phase, supporting the result of the transcription activity. These results suggested that the Pr22 gene product may be essential to cell proliferation and related to the regulation of cell cycle.

PS-9 TRANSCRIPTIONAL REGULATION BY POU HOMEODOMAIN PROTEINS

W. HERR, Rajeev Aurora, Michele Cleary, Gokul Das, Jiann-Shiun Lai, Seth Stern, Masafumi Tanaka, William Thomann, Kanna Visvanathan, and Angus Wilson, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

Many genes that control development encode sequence-specific DNA binding proteins that regulate transcription, such as those of the homeodomain class. Frequently, homeodomain proteins display quite similar DNA binding specificities but effect very different developmental programs. To understand the mechanisms by which homeodomain proteins can bind to the same DNA sequence and yet differentially regulate transcription we study transcriptional activation by the mammalian octamer-motif (ATGCAAAT)-binding proteins Oct-1 and Oct-2. These two proteins are closely related POU-type homeodomain proteins. The ubiquitously expressed Oct-1 protein is implicated in regulation of histone H2B genes and snRNA genes transcribed by either RNA polymerase II (e.g. U2 snRNA) or RNA polymerase III (e.g. U6 snRNA). In contrast, the lymphoid Oct-2 protein is implicated in cell-specific regulation of typical mRNA promoters such as the immunoglobulin gene promoters.

The promoter specificity of transcriptional activators has generally been thought to be conferred by the DNA-binding domain, which brings the activation domain to the appropriate promoter sequence. Oct-1 and Oct-2, however, can differentially activate transcription not through DNA binding specificity but instead through the use of promoter-selective activation domains which result in selective activation of snRNA promoters by Oct-1 and mRNA promoters by Oct-2. Oct-1, however, can gain the ability to activate an mRNA promoter by association with the herpes simplex virus (HSV) transactivator VP16 in a multiprotein-DNA complex containing a second host cell factor called HCF. VP16 contains a potent acidic mRNA activation domain that effectively converts Oct-1 into an Oct-2-like activator. Mechanisms by which these different transcriptional activation properties are achieved will be described.

PS-10 Glenn Galau
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NO ABSTRACT SUBMITTED

PS-11 Interests and Ethical Limits of Human Fetal Cells in Medical Research. M. ADOLPHE, Laboratory of Cellular Pharmacology, 15, rue de l'Ecole de Médecine, Paris 75006, FRANCE.

Human fetal cells have been used at the beginning of cell culture since polio vaccine development in the 1950's involved culture of human fetal kidney cells. There is no need to prove the results in using human fetal tissues for viral vaccines production. In biological research, we can also note many developments. For example, in 1961, Hayflick and Moorhead, in Philadelphia described the limited life span of human fetal cells and this discovery did allow numerous researches on in vitro ageing. On the other hand, human fetal cells are used in prenatal diagnosis. More recently, fetal transplantation progresses emphasize the discrepancies between the interests and the ethical limits in using human fetal tissues. Indeed, fetal cells have some properties that make them therapeutically useful : they proliferate and grow rapidly, they are able to undergo differentiative programm and present a reduced antigenicity compared to adult tissues. Furthermore, animal studies indicate that various diseases may respond to fetal cells transplants such as Parkinson's disease, Alzheimer dementia, insulin dependent diabete, immunodeficiency disorders... Although the treatment on human beings composed of fetal tissues transplantation have had ambiguous results, ethical problems due to the potential use of human tissue transplantation have occasioned intensive debates and provoked all over the world advisory committee to set ethical guidelines in fetal tissue research. However, deep division based on the perception of the origins of human life persist although our domination on this becomes evident. After having "dominated" the animal, man is beginning to "dominate" man. But must we or can we stop such experiments that could provide a benefic therapy for numerous human patients, that is the question.

PS-12 Biosafety, Research and Policy Challenges for Agricultural Biotechnology. A.L. YOUNG and M.K. Cordle, Office of Agricultural Biotechnology, Rm 1001 RPE, United States Department of Agriculture, Washington DC 20250-2200.

In the 1990's, society as a whole -- along with agriculture in particular -- is facing rapid political and economic change, increased international competitiveness and growing environmental concerns such as water quality, human health and nutrition, global climate change, and food safety. Biotechnology offers a powerful yet precise set of new tools to help address these issues. The last 10 years of research and development have led agricultural biotechnology to the very brink of commercialization. A strong, successful biotechnology effort requires three basic support activities: research funding, regulation and communication. The President's Budget for FY 1993 proposes $4.03B for biotechnology, an increase of $271M over FY 1992.
Research priorities include bioremediation, biofuels and agriculture. The regulatory framework for biotechnology must be based on sound scientific principles in which oversight is commensurate with the level of risk. We must communicate to the public that science is being pursued safely and that the products of biotechnology, like products of other technologies, meet the accepted regulatory criteria.

PS-13 Kenneth Olden
National Institute for Environmental Health Sciences, USA

Correspondence Between In Vivo and In Vitro Systems in Environmental Health Sciences

NO ABSTRACT SUBMITTED

I-1

Tick Cell Culture.

J.ŘEHÁČEK

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The need of appropriate media for cultivation of tick-borne pathogens stimulated the establishment of tick tissue culture /TCC/. These cultures have been derived from ixodid but not from argasid ticks. Developing adults in molting nymphs and embryonated eggs were the source for cell lines, these were not developed from organs of adult ticks and hemocytes. Leibovitz L-15 medium with supplements became the medium for TCC. TCC are applicable to various morphologic and biochemical studies, but their major application is in microbiological research. They may be useful in the isolation of various microorganisms, in studies of their properties, antigenicity, strain variation, growth cycles and mechanisms of pathogenicity at the cellular level. TTC could provide attenuated strains of pathogens with vaccine potential. TTC might be a source of antigens which can be used for immunization of animals against tick infestation. Limiting use of the application of TTC involve the failure of developing TTC from ticks which are vectors of particular pathogens, lack of cytopathic effect when infected with viruses failure of cloned cell lines and problems connected with their mass cultivation.

I-2 Leafhopper Cell Culture. Donald L. NUSS, Department of Molecular Oncology & Virology, Roche Institute of Molecular Biology, Nutley, NJ 07110

The impetus for originally establishing leafhopper cell culture systems was for use in the study of leafhopper transmitted plant viruses. Such cell systems were shown to support the replication of certain persistently transmitted viruses and provided a powerful alternative to insect vectors and plant hosts for the study of viral replication. Initial applications were practical in nature. For example, the development of a fluorescent cell-counting technique for measuring virus infectivity on cultured vector cell monolayers reduced the time required to titrate viral preparations from several months to several days. More recently, leafhopper cell lines have been successfully employed to begin studying molecular aspects of viral transmission, multiplication, assembly, cytopathology, persistence, and gene expression. In this regard, the development of new technologies has even further enhanced the utility of cultured vector cells. For example, cloning and nucleotide sequence analyses of plant reovirus genomes have revealed interesting new perspectives on the evolutionary relationship of members of this virus group. The availability of cultured cell lines derived from different leafhopper species that serve as selective vectors for specific members of this virus group provides unique opportunities to test these relationships and to investigate the basis of vector specificity. It is hoped that an examination of the past, present and projected research applications of cultured leafhopper cells will stimulate wider interest in these useful cell culture systems.

I-3

Low Cost Invertebrate Cell Culture Media.

J. L. VAUGHN. Insect Biocontrol Laboratory, ARS, USDA, Beltsville, MD, 20705.

Successful insect cell culture began in media containing mixtures of inorganic salts, amino acids, vitamins, organic acids, and sugars. The early media were supplemented first with insect hemolymph and later with fetal bovine sera (FBS). These media were costly and the serum supplements often in short supply. To produce viruses for insect control requires media that are simple to prepare, contain inexpensive, and easy to obtain ingredients. Chemically defined media developed thus far have been too costly for this purpose. One frequent substitution is an ultrafiltrate of yeast, e.g., TC-Yeastolate, for the vitamins. Hydrolysates of lactalbumin, casein, liver, and meat have been substituted for the amino acids. In many media the organic acids have been omitted or reduced in amount. Glucose has replaced the three sugar combination used in the early media. Lipids are of critical importance, for good cell growth and for virus replication. The medium we use contains an inorganic salt base, glucose as the carbohydrate source, yeastolate, two meat protein hydrolysates (Primatone-RL and tryptose) and a commercial lipid preparation. In addition, cystine, methionine, inosine, and choline chloride are added. This medium requires no supplements. A clone (Cl-15) of the Spodoperta frugiperda cell line, IPLB-Sf-21AE, will grow to a final cell density exceeding $8x10^6$ per ml in suspension culture in this medium. The ingredients for this medium are less than $2.00 per liter.

I-4 From Shake Flask To Industrial Fermentor: Challenges Of Large Scale Insect Cell Culture.

B.W. BELISLE, E.L. WALLS, C.Celeri, C.Knoch, V.Singer, K.Tang, American Cyanamid Co., San Leandro, CA 94577.

Insect viruses show good potential for use as species specific biocontrol agents in an integrated pest management program. Currently most viruses considered for pesticide use are members of the Baculovirus family, and are produced in vivo using the larvae of target insects. One of the major challenges of producing baculoviruses in vitro will be the cost effective production of unprecedented amounts of biological material to meet potential markets. An analysis of the production of insect viruses in large scale cell culture indicates there are several key areas where development effort is needed. The primary concerns are media, virus stability, culture strategy for large scale insect cell reactors, and cell and virus selection. We have begun a development effort aimed at addressing some of these concerns. Using a commercially available serum-free medium tailored to suit individual cell line requirements, we are examining the small and large scale production of extracellular and occluded LdMNPV and AcMNPV in cell culture in 5 lepidopteran cell lines. Using AcMNPV in Sf9 cells as a model, we are able to achieve cell growth and virus production at scales ranging from 100 mls to 40 liters, with cell densities and viabilities reaching those reported for cells grown in serum based medium. By applying our experience with AcMNPV production, we are able to demonstrate similar results using LdMNPV in 3 cell lines, with occlusion body production equal to or greater than that seen in cells grown in the presence of serum. We achieved these results in a variety of cell culture and bioreactor systems, including T-flask, shake flask, sparged spinner, and airlift bioreactor. Preliminary bioassays and small field trials suggest material produced in this manner is equal in efficacy to material produced in vivo.

I-5 Variability of transfection efficiency among *Drosophila* cell lines

G. Echalier, U.A. 1135 CNRS-Université P.-et-M.-Curie, Paris

As it has been well documented in mammalian cells, considerable differences exist between *Drosophila* cell lines in their ability to be transfected and to express exogenous genes.

Because of its theoretical interest and practical implications, the various aspects of this problem will be discussed.

I-6 Functional studies of ecdysone response elements by transfection of *Drosophila* Kc cells.

Both transient and stable transfections of Kc cells have been used to characterize ecdysone response elements (EcREs). We shall describe a variety of approaches we have used to identify and describe cis-and trans-acting factors which interact with EcREs in Kc cells.

I-7 Regulation by *Drosophila* segmentation genes *in vivo* and in tissueculture cells. JÄCKLE H., CAUER F., HOCH M., GERWIN N., Abt. Entwicklungsbiol., Max-Planck-Inst. f. biophysikalische Chemie,GOTTINGEN(FRG)

Genetic and molecular studies suggest that most of the genes involved in segmentation of the *Drosophila* embryo are transcription factors, some of which may act both as positive and as negative regulators of transcription on subordinate segmentation genes. We have examined the function of cis-acting regulatory elements of gap genes (which stand at the first level of the zygotic segmentation gene hierarchy) and their requirements for transacting factors. *In vivo* experiments suggest that activation and the spatial domain of gap gene expression is regulated by the combined action of activators and repressors which act in a concentration-dependent manner. Using the *Drosophila* Schneider cell line system we have examined the regulatory potential of *Krüppel*, which encodes a zinc finger-type protein expressed in a central position of the blastoderm embryo. Using a single 11-bp Kr *in vitro* binding site common to several putative Kr target genes we show that low amounts of *Krüppel* expression leads to transcriptional activation whereas high amounts result in repression suggesting that *Krüppel* itself can act both ways : as a concentration-dependent positive and negative regulator of transcription. A mechanism by which developmental genes can be regulated in response to such factors will be presented.

I-8 Co-activators Associated with TATA-binding Protein Mediate Transcriptional Activation in *Drosophila*.

DYNLACHT B.D., HOEY T., WEINZIERL R. and TIJIAN R., Howard Hughes Medical Institute, Department of Molecular and Cell Biology, University of California, BERKELEY, Ca94720

A key step in the regulation of transcription involves interactions between promoter-selective factors and various components of the transcriptional apparatus. Here we describe the requirements for transcriptional activation directed by NTF-1, a developmentally regulated transcription factor in *Drosophila*. Reconstituted transcription reactions carried out with fractionated basal factors isolated from *Drosophila* embryos reveal that activation by NTF-1 requires factors present in the endogenous TFIID fraction which are distinct from the purified *Drosophila* TATA-binding protein (dTBP). Two types of analysis, glycerol gradient sedimentation and immunoprecipitation, reveal that endogenous TFIID is a multi-protein complex containing TBP and a least six distinct, tightly associated polypeptides which we have termed TBP-associated factors (TAFs). Preparations of TBP separated from TAFs by fractionation with denaturants no longer support activation by NTF-1 but retain basal level activity. Addition of immunopurified TAFs to free TBP restores the ability of NTF-1 to activate transcription without influencing basal transcription. These results suggest that one or more of the TAF polypeptides confers co-activator function. Recently, we have isolated cDNA clones for several of these TAFs. We present results describing the DNA sequence and structural analysis of these clones.

I-9 Expression and Regulation of 1731, a *Drosophila* retrotransposon.

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1731, a *Drosophila* retrotransposon, is 4648 bp long and formed by two Long Terminal Repeats (LTRs), having U3-R-U5 architecture, which frame a *gag* gene and a *pol* gene coding, as we demonstrated, for a functional reverse transcriptase.

Transfections in cultured *Drosophila* cells show that the 3' or 5' LTRs are unidirectional promoters which combine silencer and enhancer effects; the last one, due to 28 bp of the U3 region, is also required for the negative regulation by ecdysteroids and positive regulation by heat-shock.

By co-transfections with antisense constructs, we decrease the translation of the gag and pol gene messengers.

Experimental data point out the crucial role of the U3 region which is a target for topoisomerases II and, moreover, binds specifically several proteins (among which a "Nuclear single strand Binding Factor (NssBF).

The combined functions of all these factors will be discussed.

I-10 **Viral Evolution and Insects as a Possible Virological Turning Table**

H. KOBLET, Institute of Medical Microbiology, University of Berne, CH-3010 Berne, Switzerland

The classification of viruses (Arch.Virol. Suppl.2, Springer,1991) comprises 2430 viruses belonging to 73 families or groups. For practical reasons the viral families are distinguished by host (invertebrates, vertebrates, plants etc.); having or not an envelope; type of genome (DNA/RNA); number of strands (double- or single-stranded) and structure of virion (isometric, pleomorphic or rodlike). However, the more viral genomes become sequenced, the more it will be possible to create a phylogenetic taxonomy of viruses only partially coinciding with the classical boundaries. In case of viruses with RNA genomes, familiar relations based on comparison of sequences allowed the construction of phylogenetic trees (Ahlquist et al., 1985; Argos, 1988; Habili and Symons, 1989; Goldbach, 1987, 1990; Strauss et al., 1990; Bruenn, 1991; Gorbalenya et al., 1991; Koonin, 1991). Superfamilies emerge ("Sindbis-" or Tobamo-; "Picorna-" or Poty-like etc.) with a host range encompassing without exception plants, vertebrates and insects. Insects seem to have played a major role in transmitting viruses either to plants or vertebrates. This point stresses cell culture work. Possible reasons for evolution of RNA genomes will be given.

I-11 Insect Transmitted Plant Viruses.

R. Creamer. Dept. of Plant Pathology, Univeristy of California, Riverside, CA 92521

Most plant viruses are naturally transmitted by insects. The insects are predominantly homopteran, with the largest number of plant viruses transmitted by aphids, and fewer plant viruses transmitted by leafhoppers, planthoppers, and whiteflies. Insect transmission is usually grouped by viral persistence in the vector, or, if known, part of the insect with which the virus is associated. Increased persistence of the virus is characterized by a closer association between virus and insect vector. Non-persistently transmitted (stylet borne) viruses, such as potyviruses, have been associated with the stylets of their aphid vectors. Viruses such as closteroviruses and maize chlorotic dwarf virus, which are transmitted by aphids and leafhoppers, respectively, show semipersistent or foregut-borne transmission. Persistently transmitted viruses are characterized by a closer association with their vectors. These viruses have been separated into those that circulate in the vector, and those that circulate and replicate in their insect vector. Persistently circulative viruses include geminiviruses, transmitted by whiteflies and leafhoppers and luteoviruses, transmitted by aphids. Specific membrane receptors in the walls of the gut and salivary glands of their vectors recognize these viruses. Persistently propagative transmitted viruses include rhabdoviruses, reoviruses, and tenuiviruses, transmitted by planthoppers, and marafiviruses, transmitted by leafhoppers. This type of transmission necessitates an integral association between insect and virus. These viruses are transstadial and many are transovarially transmitted. Insect cell culture has been used for the study of a few propagative plant viruses, specifically reoviruses and rhabdoviruses using leafhopper culture. Insect cell culture could provide an essential tool for the study of insect transmission, with particular focus of the interaction between a plant virus and its insect vector.

I-12 The study of Alpha togaviruses in cultured mosquito cells.

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By studying the replication of Sindbis virus in an established line of mosquito cells (*Aedes albopictus*), we have been able to observe mutant viral phenotypes, which would not have been evident had we studied replication only in vertebrate cells. For example, because the replication of standard Sindbis virus in mosquito cells is sensitive to methionine deprivation, we were able to isolate a mutant virus SV_{1m21}, which is resistant to methionine deprivation. By mapping the causal mutation for resistance to methionine deprivation, we were able to associate a specific nonstructural protein encoded by Sindbis virus with the methylation of the 5' terminal cap of the viral RNA. Studies of viral replication in mosquito cells also enabled us to isolate host range mutants of Sindbis virus; in one case these mutants were able to replicate in mosquito cells but were restricted in vertebrate cells, and in the other case they were able to replicate in vertebrate cells but were blocked in mosquito cells. Examples of such mutants will be described. Ultimately the study of arthropod-borne viruses in cultured cells derived from the appropriate vector (e.g.; mosquito) should help us understand the replication of these viruses in the intact invertebrate host.

I-13 Insect Transmitted Vertebrate Viruses: Flaviviridae

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The Flaviviridae include over 60 viruses, nearly half of which have been associated with human disease. These viruses are among the most important arthropod-borne viruses world-wide and include dengue, yellow fever, and Japanese encephalitis viruses. Morbidity and mortality from these viruses are variable, but account for millions of encephalitis, hemorrhagic fever, arthralgia, rash, and fever cases per year. Most of the members of this family are transmitted between vertebrate hosts by arthropod vectors, most commonly mosquitoes or ticks. Transmission cycles can be simple or complex depending on the hosts and vectors involved, the virus, and the environmental factors affecting both the hosts and the virus. Replication of virus in invertebrate hosts does not appear to result in any significant pathology, suggesting a close evolutionary relationship between virus and vector. Such relationships are reflected by growth of these viruses in invertebrate culture where replication usually results in a steady state, persistent infection without cytopathic effect. Yields of virus from insect tissue culture are variable but are generally similar to yields in vertebrate cells. Replication kinetics are comparable between insect and vertebrate cell lines despite differences in incubation temperature. Both vertebrate and insect cell culture systems continue to play a significant role in flavivirus isolation and the diagnosis of disease caused by these agents. Additionally, these culture systems permit the study of flavivirus attachment, penetration, replication and release from cells and have been instrumental in the production and characterization of live-attenuated vaccines. Both vertebrate and insect tissue culture systems will continue to play a significant role in basic and applied flavivirus research in the future.

I-14 Insect Transmitted Vertebrate Viruses: Bunyaviridae.

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The Bunyaviridae family was established in 1975 to encompass a large group of arthropod-borne viruses sharing morphologic, morphogenic, and antigenic properties. More than 250 serologically distinct members comprise the Bunyaviridae. Four animal virus genera, *Bunyavirus*, *Hantavirus*, *Nairovirus*, and *Phlebovirus*, and one plant virus genus, *Tospovirus*, have been defined within the family. Prototype viruses for each respective genus are: Bunyamwera, Hantaan, Crimean-Congo hemorrhagic fever, sandfly fever Naples, and tomato spotted wilt viruses. Most viruses in the family have been isolated from or are transmitted by arthropods. Hantaviruses are exceptions; these viruses are primarily rodent-borne and have no known arthropod vector, but instead are transmitted by aerosolized rodent excreta. Many members of the Bunyaviridae have been associated with severe or fatal human diseases such as Korean hemorrhagic fever, Rift Valley fever, Crimean-Congo hemorrhagic fever, and La Crosse encephalitis. Viruses in all genera except the *Hantavirus* genus are capable of alternately replicating in vertebrates and arthropods, generally are cytolytic for their vertebrate hosts, but cause little or no cytopathogenicity in invertebrate hosts. Similarly, with the exception of hantaviruses, which readily establish persistent, noncytolytic infections in mammalian cell cultures, viruses in this family generally cause lytic infections of cultured vertebrate cells but elicit little detectable cytopathology in insect cell cultures. The replicative properties of Bunyaviridae in cultured invertebrate and vertebrate cells will be discussed. Also, data will be presented concerning the use of eucaryotic expression systems, such as vaccinia virus and baculovirus, in cultured cells for the study of isolated genes of these viruses.

JS-1 Transcriptional Regulation of Pathogenesis by the Moloney Murine Leukemia Virus. N.A. SPECK[1], S. Wang[1], I. Cherepennikova[1], Y.H. Hsiang[2], and D.H. Raulet[2]. [1]Department of Biochemistry, Dartmouth Medical School, Hanover, NH, 03755-3844; and Department of Molecular and Cell Biology, University of California, Berkeley, CA, 94720.

The Moloney murine leukemia virus causes a thymic leukemia in mice. The transcriptional enhancer of the Moloney virus is a major genetic determinant of its thymic disease specificity. Several protein binding sites in the Moloney virus enhancer contribute to this thymic disease specificity, including the Leukemia virus factor b (LVb) site, and an adjacent "core" sequence. Point mutations introduced into either of these sites cause a significant shift in the disease specificity of the Moloney virus from thymic to erythroid leukemias. We have purified proteins from calf thymus nuclei that bind to the core site in the Moloney virus enhancer. These core-binding factors (CBF) appear to be expressed primarily in hematopoietic cells. Selected and amplified binding site analysis indicates that CBF preferably binds the recognition sequence PyGPyGGTT. CBF also binds to core sites in the T cell receptor γ and δ chain genes. The core sites in these enhancers have been identified as critical cis-acting sequences for transcription of the T cell receptor γ and δ chain genes. Therefore, we believe that CBF may be important both for determining the thymic disease specificity of the Moloney virus, and also for the transcription of genes that are specifically expressed in T cells.

JS-2 **Regulation of Gene Expression by the Nuclear Hormone Receptor Superfamily** D. D. MOORE, Dept. Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

The receptors for the steroid hormones, thyroid hormone (T3), vitamin D and retinoic acid (RA) are all members of a related superfamily of nuclear transcription factors. These proteins specifically bind both their ligands and DNA sequences near regulated target genes. The DNA binding specificity of monomeric forms of these receptors is determined by a short region within the highly conserved DNA binding domain. Unexpectedly, the members of this large superfamily can be divided into only two major groups based on the sequence of this specificity determining region, called the P box. For example, the P box sequences of the T3 receptors and the RA receptors are identical. A number of additional superfamily members that do not bind known hormones or ligands also share this sequence identity. Within each of the major groups, there is some overlap in target gene activation, even by receptors for ligands that show quite different biological regulatory effects. However, a number of mammalian genes cells are regulated by only T3 or RA. This talk will discuss several approaches to understanding the mechanisms that allow members of this subgroup to regulate distinct sets of target genes.

JS-3 Regulation and Function of the *E75* Ecdysone-Inducible Gene of Drosophila. W.A. SEGRAVES. Howard Hughes Medical Institute, Gene Expression Lab, The Salk Institute, La Jolla, CA 92037

The insect molting hormone ecdysone induces genetic regulatory hierarchies manifested by the induction of polytene chromosome puffs in the larval salivary gland. It has been proposed that the products of the transiently active early ecdysone-inducible genes regulate late ecdysone responses including the repression of early ecdysone-inducible genes and the induction of late ecdysone-inducible genes.

The early ecdysone-inducible *E75* gene responsible for the 75B puff consists of three overlapping transcription units which are expressed in a variety of tissues and developmental stages, encoding three different evolutionarily conserved proteins which are also members of the steroid receptor superfamily. Identification and analysis of flies carrying mutations in *E75* demonstrates that these proteins have distinct genetically separable roles in the regulation of larval molting and metamorphosis.

In transfection studies of cultured cells, the major larval E75 protein, E75 A, is capable of hormone-independent repression of its own promoter. This protein may thus be one of the early ecdysone-inducible products responsible for the secondary repression of early genes. Regions of the E75 A protein required for repression have been identified, as have autoregulatory response elements and ecdysone response elements in the E75 A promoter. Despite marked similarities in the DNA binding domains of E75 and the ecdysone receptor, these proteins appear to have distinct DNA binding specificities and to act through separate elements.

The similarity between the E75 proteins and members of the steroid receptor superfamily within all highly conserved regions of the hormone binding domain suggests that the E75 proteins are receptors. Nonetheless, the putative E75 hormone binding domain is not activated by known steroid, thyroid, retinoid or insect juvenile hormones, suggesting that the E75 ligand is a novel hormone which might act through E75 to modulate ecdysone response. The finding in Drosophila and other invertebrates of a growing number of receptor-like molecules for which no ligand has been identified suggests that there may be many undiscovered invertebrate hormones.

JS-4 Molecular Biology of Ecdysone and Juvenile Hormone Action.

E.M. Berger, Biology Dept., Dartmouth College, Hanover, NH 03755

The development of holometabolous insects is regulated through the interaction of two hormones; ecdysterone, the steroid moulting hormone, and juvenile hormone, a sesquiterpene. In an effort to understand the mechanisms by which these hormones modulate the transcriptional activity of target genes a hormone responsive, Drosophila, continuous cell culture system has been developed and analyzed using DNA mediated gene transfer and a transient transfection assay.

The small heat shock protein genes are inducible in response to either high temperature or ecdysterone treatment, and their expression shows a parallel pattern of regulation in vivo during imaginal disc development. Regulatory sequences required for both heat shock and ecdysterone induction are located in the 5' untranscribed region of each gene. The heat shock regulatory elements are composed of a head to head array of redundant elements (HSEs) containing the basic motif NGAAN. The nature and location of ecdysterone response elements (EcREs) is less certain but the EcREs appear to contain a 15bp dyad in which two 7bp half-elements (TGANCPyPy) are separated by a single base pair spacer. The EcRE is the binding site for the ecdysterone receptor. Binding of a ligand free receptor to the EcRE leads to transcriptional repression, and the addition of ecdysterone and its binding to the receptor, converts the complex into a transcriptional activator.

The juvenile hormone analogue, methoprene, inhibits the ecdysterone inducible expression of small hsps, but has no effect on the expression following heat shock. Models of methoprene action are discussed.

JS-5 Biogenesis of Secondary Cell Walls of Flowering Plants, C.H. HAIGLER, Department of Biological Sciences,Texas Tech University, Lubbock, Texas 79409 USA

Secondary cell walls, which can be thought of as extensive extracellular matrices, are important agents of differentiation in flowering plants. Deposition of these thick walls by some cells confers specialized functions including: water conduction (tracheary elements); gas exchange (stomates); protection and seed dispersal (trichomes); and structural reinforcement (fibers and sclerenchyma). An overview will be presented on the composition and biogenetic mechanism of secondary walls with emphasis on experimental data obtained from tracheary elements and cotton fibers differentiating in tissue culture. The complexity of the biogenetic process, which involves many cellular components, and the implications of the finished extracellular matrix for cell differentiation both indicate the necessity of tight integration of secondary wall deposition with the overall scheme of plant development. Furthermore, it is likely that the forming extracellular matrix itself exerts control over the general scheme of development. Evidence for the latter possibility derived from experiments on secondary wall deposition under pharmacological and environmental stress will be presented. The research to be discussed was supported by grants from Cotton Incorporated, the NSF Developmental Biology Program, and the Texas Advanced Technology Program.

JS-6 Structural Models of Primary Cell Walls of Flowering Plants. N.C. CARPITA and D.M. Gibeaut. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907 USA

Advances in polymer chemistry and preservation of structure for electron microscopy provide the best view to date of how polysaccharides and structural proteins are assembled into plant cell walls. The walls that form and partition dividing cells differ chemically and structurally from the wall that expands to provide a cell with its functional form. In grasses, the chemical structure of the wall differs from those of all other flowering plant. Nevertheless, both types of walls must conform to the same physical laws. Cell expansion and elongation must occur via the strictly regulated reorientations of each of the walls components that first permit the wall to stretch in specific directions and then lock into final shape. Information now available on the chemical structure of individual polymers will be integrated with that from new approaches to probe the arrangement of the polymers within the walls of individual cells. From this integration of information, we provide structural models of two distinct types of walls in flowering plants consistent with the physical properties of wall and its components. Each model also accounts for unique alterations in chemical and physical structure that accompany cell expansion. The interaction of plasma membrane and cell wall via vitronectin- and fibronectin-like proteins will also be discussed.

JS-7 **Extracellular Matrix and the Control of Cell Migration**: S.L. SCHOR[1], I. Ellis[1], A.M. Schor[2], and A.M. Grey[1] 1. Dept of Cell and Structural Biology, Stopford Building, University of Manchester, Manchester M13 9PT (England) 2. Dept of Medical Oncology, Christie Hospital, Wilmslow Rd, Manchester M20 9BX (England)

The extracellular matrix (ECM) plays a central role in the control and integration of cell migration in multicellular organisms. This is accomplished by regulatory mechanisms operative at several different levels, including the supramolecular (where the packing and orientation of fibrillar components of the matrix impose physical constraints on the directionality of movement) and the cellular (where interactions of matrix macromolecules with cell surface receptors affect cell adhesion to the substratum and may mediate a haptotactic response). Apart from these two well-recognized mechanisms, the ECM may also affect cell migration by (a) modulating cellular response to cytokines, and (b) soluble fragments of matrix macromolecules acting in a cytokine-like fashion. Our own work in this area has indicated that the effect of TGF-β1 on fibroblast migration is modulated by the presence of a collagenous matrix: TGF-β1 stimulates cell migration when assessed in Boyden chamber assays, but inhibits migration into 3D lattices of type I collagen fibers. Data will also be presented indicating that extremely low concentrations (pg/ml) of certain soluble fibronectin fragments exert a profound stimulatory effect upon cell migration into 3D collagen matrices. These particular fragments do not affect fibroblast migration when assessed in Boyden chamber assays, again indicating the important regulatory role played by the ECM.

JS-8 **Direct Interaction of Heparin with Heparin-Binding (Fibroblast) Growth Factor (HBGF)-Receptor Mediates Ligand Binding.** M.KAN, J. Hou, J. Xu, E. Shi, F. Wang, G. Yan and W.L. McKeehan W. Alton Jones Cell Science Center, 10 Old Barn Road, Lake Placid, NY 12946

The extracellular matrix (ECM) plays important roles in cellular proliferation and differentiation. A part of the effect is mediated by ECM-bound growth factors. HBGFs bind to heparin-like sites on cells or ECM in addition to transmembrane receptors and a role of the site in modulating the binding to the receptor has been suggested. We developed an HBGF binding assay to analyze the effect of heparin and ECM components using isolated recombinant HBGF-R (flg) expressed by the baculovirus-insect cell system. Heparin strongly enhanced both HBGF-1 and HBGF-2 binding to the isolated receptor. Preincubation of receptor and heparin was as effective as soluble heparin and the heparin effect was abolished above 0.5 M NaCl. The heparin binding site on the receptor was localized by both biochemical analysis and site-directed mutagenesis. A synthetic polypeptide containing the heparin-binding sequence competed with both HBGF-1 and -2 binding. HBGF binding to heparin-like sites on cells was released by trypsin treatment and the solubilized cell surface extract substituted for heparin. These results suggest that ECM heparin-like sites directly interact with and modify activity of HBGF receptors.

JS-9 The Commercialization of Insect Resistant Cotton-The Road From the Lab Bench to the Market-Place. F. J. PERLAK. Monsanto Company, 700 Chesterfield Village Parkway, Chesterfield, MO 63146.

Insect resistant cotton, genetically modified by the introduction of a gene encoding an insect control protein from the bacterium Bacillus thuringiensis, is scheduled for commercialization in the mid-1990's. Monsanto's research program over the last seven years has made substantial progress toward the realization of this commercial objective. Technical hurdles such as plant transformation limitations and low insect control protein levels were overcome resulting in cotton plants which were resistant to insect damage at levels equivalent to or better than those achieved with chemical insecticide applications. Successful commercialization will depend on regulatory approval and the incorporation of this exciting technology into integrated pest management systems and existing agricultural practices.

JS-10 Searching for Insect Resistance Genes: Alternative Sources and Methods. L. L. MURDOCK, Department of Entomology, Purdue University, W. Lafayette, IN 47907 USA

Transgenic crop plants expressing insect resistance genes have the potential to become widely-used pest management tools for controlling field and storage pests. However, the supply of known resistance genes is quite limited. In addition to novel *Bacillus thuringiensis* protoxin-like genes – the protein products of which are specifically and highly toxic to insects – we need to seek genes which code for products that repel insects, deter feeding, or retard growth and reproduction. Examples include genes for α-amylase inhibitors, proteinase inhibitors, and lectins. Such genes, which may be termed biopestistatic rather than biopesticidal, can be both effective and durable, especially if used in combination. A key question about resistance genes is whether they can prevent economic pest populations from developing in the field: promising genes may be overlooked if we screen only for acute toxicity toward individual insects in short-term experiments. New methods are needed as well to speed screening and evaluation of candidate genes. One possible early evaluation tool involves rearing pest insects on callus tissue expressing candidate resistance genes.

JS-11 Characterization of Insect Brush Border Proteins Specific to Bacillus thuringiensis Insecticidal Proteins by Toxin and Monoclonal Antibody Binding. M. Adang, S. Paskewitz, and S. Garczynski. Dept. of Entomology, University of Georgia, Athens, Georgia 30602.

Bacillus thuringiensis insecticidal toxins recognize proteins located in midgut epithelia of target insects. B. thuringiensis CryIA(c) toxin binds to membrane vesicles from sensitive and, unexpectedly, tolerant insects. Protein blots showed that 125I-CryIA(c) toxin binds to a 120 kDa protein in Manduca sexta membranes (Garczynski, Crim, and Adang, 1991, Appl. Environ. Microbiol. **57**, p. 2816-2820). Microgram amounts of M. sexta 120 kDa protein were spotted onto nitrocellulose, implanted into mice and, after hybridoma fusions, five monoclonal antibody cell lines selected. A partial characterization indicates that three antibodies are non-specific and may react to carbohydrate determinants. One antibody reacts to M. sexta 120 kDa and 100 kDa peptides, and cross-reacts to specific toxin-binding proteins in Heliothis virescens and Helicoverpa zea. These results suggest that certain B. thuringiensis toxin binding proteins are immunologically related between insects of different genera.

JS-12 Baculovirus-mediated Expression of an Insect-specific Scorpion Toxin. P.V. CHOUDARY, B.F. McCutchen, E. Fowler[1], B.D. Hammock and S. Maeda. Dept. Entomology, Univ. California, Davis, CA, and [1]CIBA-GEIGY Ag. Biotech Unit, Research Triangle Park, NC

We have constructed a recombinant baculovirus, vAcUW2(B).AaIT, carrying a synthetic gene encoding an insect-specific scorpion toxin under the transcriptional control of the baculoviral p10 promoter. Cultured insect cells, upon infection with this recombinant virus, produced biologically active AaIT. The signal sequence appeared to be appropriately cleaved off, resulting in the mature AaIT protein. Susceptible larvae, upon infection with the recombinant virus, showed symptoms, characteristic of AaIT neurotoxicity, viz., sodium channel blocking, and succumbed significantly faster than did the larvae infected with wildtype baculovirus. The results demonstrate the feasibility of expressing insecticidal proteins in baculoviruses to increase their speed of insect kill and to reducing the insect feeding damage. Recombinant baculoviruses with enhanced speed of kill will be valuable as effective alternatives to chemical insecticides.

JS-13 Properties and Analysis of Insecticidal Bacterial Proteins Produced in Lepidopteran Cell Cultures Using Baculovirus Vectors. B. A. FEDERICI, Department of Entomology, University of California, Riverside, CA 92521.

Full length (72K) and truncated (61K) CrylVD mosquitocidal proteins of *Bacillus thuringiensis* (Bt) were expressed in *Spodoptera frugiperda* cells and larvae of *Trichoplusia ni* using a baculovirus vector to determine the utility of the baculovirus/lepidopteran system for expressing Bt proteins with a non-lepidop-teran toxicity as well as to investigate the role of CrylVD peptides in toxicity. The CrylVD genes were inserted into the *Autographa californica* multinucleocapsid nuclear polyhedrosis virus (AcMNPV) under control of the polyhedrin promoter by recombination in *S. frugiperda* cells between a transfer vector carrying the Bt genes and vDA26Z, a recombinant AcMNPV carrying the *Escherichia coli B*-galactosidase gene under control of the DA26 promoter. Recombinant AcMNPVs carrying the genes were detected as blue occlusion body-negative plaques in monolayers of *S. frugiperda* cells grown in the presence of X-Gal. Infection of *S. frugiperda* cells and *T. ni* with plaque-purified recombinant virus expressing either the full length or truncated CrylVD protein resulted in the synthesis of proteins of the expected size, confirmed by immunoblot analyses, and their crystallization into cuboidal inclusions in the cytoplasm. Infected cells and purifieid inclusions from the virus (AcCrylVD) expressing the full length protein were highly toxic to mosquito larvae, but similar preparations from the virus (AcCrylVD-C) expressing the truncated protein were non-toxic. Proteolysis with trypsin of CrylVD proteins produced by Bt and the recombinant AcMNPVs yielded peptides corresponding in size, showing that synthesis of mosquitocidal Bt proteins in lepidopteran cells was competent. The lack of toxicity of the truncated CrylVD protein, which like the toxic full length protein yields a 33K protein on proteolysis that has been implicated in toxicity, indicates that by itself this protein is non-toxic. Experiments with insecticidal proteins from *B. thuringiensis* active against lepidopterous insects have shown that these proteins are also functional when produced in lepidopteran cells. These results demonstrate the utility of the baculovirus system for expression of insecticidal Bt proteins and for investigation of their mode-of-action.

P-1 Crops for Tomorrow

Indra K. VASIL, Laboratory of Plant Cell and Molecular Biology, University of Florida, Gainesville, FL 32611-0514, U.S.A.

Rapid advances in cell culture and genetic transformation of plants have resulted in the production of transgenic plants with value added and other useful agronomic traits in many important crops, such as rice, maize, wheat, soybean, oil seed rape, cotton, etc. Many of the engineered crops are currently undergoing extensive field trials to fulfil regulatory requirements, to ensure safety, and to assess field performance, and are expected to be introduced into the market place before the end of this decade.

P-2 M. Fromm
Monsanto Company

NO ABSTRACT SUBMITTED

P-3 Genetic engineering for fertility control. J. LEEMANS, Plant Genetic Systems N.V., Jozef Plateaustraat 22, 9000 Gent, Belgium.

A dominant gene for male sterility

To engineer male sterility, we isolated the tobacco TA29 gene which is characterized by its extreme cell specificity in the tapetal cells of immature anthers. The 5' regulatory region of TA29 was used to target the expression of ribonucleases such as barnase, from *Bacillus amyloliquefaciens*, specifically to the tapetum of immature anthers. Barnase expression led to the precocious degeneration of the tapetum cells, the arrest of microspore development, and male sterility.

Fertility restoration

We have constructed genes which can restore fertility to the genetically engineered male sterility. These chimaeric genes consist of a tapetal specific promoter linked to the intracellular inhibitor of barnase, called barstar. When barnase and barstar are produced simultaneously in the same tapetal cells, they form a one to one complex which has no residual ribonuclease activity. Progeny of crosses between male sterile oilseed rape plants that express barnase and fertile pollinator plants that are homozygous for the barstar gene produce fully normal pollen and showed normal seed set upon selfing.

Maintaining male sterility

To maintain and multiply male sterile plants, the male sterility gene was linked to the herbicide resistance bar. The linkage of both genes allows the elimination of segregating fertile plants after backcrossing by herbicide treatment, thereby eliminating all herbicide sensitive, male fertile plants.

Applications

The new hybrid system has been successfully applied to various crops including corn, oilseed rape, Brassica vegetables and cotton. Field tests in N. America and Europe allowed to identify male sterile oilseed rape lines in which the sterility is stable under various environmental conditions and which show otherwise normal agronomic performances. The advantages of this sytem for F1 hybrid breeding and seed production in various crops will be discussed.

P-4 Development of Crop Plants with Disease Resistance and Value Added Traits

PETER J.M. VAN DEN ELZEN, André Hoekema and Ben J.C. Cornelissen
MOGEN International N.V., Einsteinweg 97 , 2333 CB Leiden, The Netherlands.

Plant genetic engineering has enabled the development of crops with enhanced resistance to diseases and pests, as well as crops with an added value. In collaboration with academic as well as industrial laboratories, MOGEN has been active in both areas. We have developed potatoes resistant against the viruses PVX and PLRV. After four years of field trials with two different PVX resistant potato cultivars, we were able to conclude that it is possible to engineer a high level of virus resistance while preserving the intrinsic cultivar characteristics. These field trials were performed in parallel with an official variety registration procedure to investigate the possibility to obtain Plant Breeders Right Protection on transgenic cultivars.

MOGEN's fungal resistance program focusses on the exploitation of plant genes involved in the phenomenon of "induced resistance" in plants.This resistance is active against a wide variety of viral, bacterial and fungal pathogens. From tobacco we have isolated genes that are expressed at a high level during this resistance reaction. Using in vitro assays and using transgenic plants we have identified proteins with high growth inhibiting activity against chitin containing fungi like Fusarium, as well as against fungi lacking chitin like Phytophthora infestans.

Since plants are cheap producers of biomass, we have investigated the potential use of crop plants for large scale production of valuable proteins. In this program we produced authentic Human Serum Albumin in transgenic potatoes. We have also made transgenic crop plants producing high levels of different industrial enzymes. Using Bacillus licheniformis alpha-amylase, an enzyme with a wide range of applications in different industries, we have demonstrated that transgenic plants are a competitive source of active industrial enzymes. Moreover transgenic seed appeared to provide a very stable formulation of active enzyme, and can be used directly in an industrial application.

P-5 RFLP Analysis of Plant Regeneration in Maize.
C.L. ARMSTRONG[1], J. Romero-Severson and T.K. Hodges[2], Agrigenetics Co., Madison, WI, 53716. [1]Current address: Monsanto Co., St. Louis, MO, 63198. [2] Purdue University, W. Lafayette, IN, 47907.

Plant regeneration from in vitro cultures of maize is quite genotype-dependent. Many agronomically desirable lines (e.g., B73 and Mo17) regenerate poorly. The goals of this study were to: 1) determine the utility of backcross breeding for improving in vitro regeneration; and 2) identify molecular markers linked to "regeneration genes". The frequency of initiation of friable, embryogenic callus from immature B73 embryos was increased dramatically by introgression of chromosomal segments from A188. When cultured on a modified N6 medium, less than 0.2% (5/3484) of immature B73 embryos formed embryogenic callus. After crossing to A188, backcrossing to B73 for six generations with selection at each generation for high-frequency initiation of embryogenic cultures, and selfing for four generations to select homozygous lines, the average embryogenic culture initiation frequency increased to 46% (256/561). RFLP analysis revealed that five chromosomal segments from A188 were retained through at least the fifth backcross generation. The hypothesis that one or more of these five regions contains genes controlling regeneration in maize was tested using an F_2 population of the cross A188 X Mo17. A set of five DNA markers (3 of them linked) explained 82% of the observed phenotypic variance for percentage of immature embryos forming embryogenic callus. Four of the five markers were located in or near introgressed A188 chromosome segments. The region marked by probe c595 on the long arm of chromosome 9 was highly associated with several measures of in vitro culture response. We propose that there is a major gene (or genes) in this region in A188 that promotes embryogenic callus initiation and plant regeneration in B73, Mo17, and probably many other recalcitrant inbred lines of maize.

P-6 **Proteins Associated with the Induction of Brassica napus Microspore Embryogenesis.**
J.H.G. CORDEWENER, R. Busink, Y. Nöllen, J.B.M. Custers, J.A. Traas and J.J.M. Dons. Centre for Plant Breeding and Reproduction Research (CPRO-DLO), P.O. Box 16, NL-6700 AA Wageningen, The Netherlands.

Microspores isolated from flower buds of B. napus in the late uninucleate/early binucleate stage started to divide within 24 hours after initiation of the culture. At 32 °C sporophytic divisions predominated, whereas at 18 °C most divisions were gametophytic. To investigate which proteins are involved in the induction of embryogenesis microspores were cultured with [^{35}S]-Met included in the medium. Using 2-D PAGE the protein pattern of an embyrogenic culture was compared with that of a non-embryogenic culture. While most protein spots were present under both temperature treatments, some proteins appeared to be specific for embryogenesis. We also performed labellings experiments with $^{32}P_i$. Both under embryogenic and non-embryogenic conditions a large number of proteins were phosphorylated, with only minor differences between both temperature treatments. Antibodies against a set of cytoskeletal proteins and MPM-2, an antibody which recognises a mitosis-specific phosphorylated epitope, were used to identify the corresponding antigens on Western blots obtained from 2-D gels. For α- and ß-tubulin and also for actin several isotypes were detected. $^{32}P_i$ labelling demonstrated that some of the isotypes of all three cytoskeletal proteins were differently phosphorylated under embryogenic and non-embryogenic conditions.

P-7 Developmental and Molecular Genetics of Embryogenesis in Arabidopsis thaliana.
D.W. MEINKE. Department of Botany, Oklahoma State University, Stillwater, Oklahoma, 74078 USA.

The purpose of research in my laboratory has been to identify genes with essential functions during plant embryogenesis. Over 250 embryonic mutants of Arabidopsis thaliana have been isolated and characterized following either EMS seed mutagenesis or T-DNA insertional mutagenesis with Agrobacterium tumefaciens. These mutants exhibit a wide range of embryonic defects including altered patterns of symmetry, abnormal suspensors, distorted epidermal layers, twin embryos, fused cotyledons, enlarged apical meristems, reduced hypocotyls, distorted cotyledons, multiple cotyledons, giant vacuolated cells, mature endosperm tissue without cell walls, and embryos that protrude through the seed coat late in maturation. Some of these mutants are likely to be defective in genes with important regulatory functions during plant embryogenesis. Others such as a biotin auxotroph that becomes lethal during embryogenesis are defective in genes with general housekeeping functions not directly involved in the control of morphogenesis. Emphasis is currently being placed on 15-20 mutants with particularly unusual patterns of development and 25-30 mutants tagged with T-DNA following seed transformation. Plasmid rescue has been used to recover plant sequences flanking T-DNA insertion sites in several mutant families. The relationship between these studies of zygotic embryogenesis and current approaches to plant regeneration through in vitro embryogenesis will be discussed. This research has been funded by NSF and USDA grants.

P-8 SECRETED PROTEINS AS MODULATORS OF PLANT EMBRYOGENESIS.
S.C. de Vries, T. Hendriks, A.J. de Jong, M.V. Hartog, E.A. Meijer, A. van Kammen. - Department of Molecular Biology, Agricultural University Wageningen, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands.

Somatic embryogenesis in carrot is accompanied by the phytohormone - controlled expression of secreted extracellular matrix glycoproteins (De Vries et al., 1988, Genes & Dev. 2, 462-476). Some of these proteins are associated with the cell walls of non-embryogenic suspension cells (van Engelen et al., 1991, Plant Physiol. 96, 705-712), while others are exclusively synthesized by embryogenic cells (Sterk et al., 1991, Plant Cell 3, 907-921). By using the glycosylation inhibitor tunicamycin, arrested somatic embryogenesis could be rescued by a secreted 38 kDa cationic peroxidase (Cordewener et al., 1991, Planta 184, 478-486), while arrested embryo development in the mutant cell line ts11 (LoSchiavo et al., 1990, Mol. Gen. Genet. 223, 385-393) could be rescued by a single secreted 32 kDa medium protein (De Jong et al., 1992, submitted). These results demonstrate that several secreted glycoproteins are indispensable for correct somatic embryo development. Employing *in situ* localization of mRNAs encoding an embryo-secreted lipid transfer protein (LTP) an identical gene expression pattern was found in somatic and zygotic embryos, indicating that proteins secreted by somatic embryos are also involved in zygotic embryogenesis. Based on morphological examination of arrested and rescued somatic embryos, the biochemical and molecular identification of several secreted proteins and the expression pattern of the encoding genes, we propose a function for secreted proteins in the formation and maintenance of the embryo protoderm, presumably through their action on primary cell wall components (van Engelen and de Vries, 1992, TIG, in press).

P-9 The Combination of Microprojectile Bombardment and *Agrobacterium tumefaciens* Improves Plant Transformation Frequencies. D.L. BIDNEY, C.J. Scelonge and J. Malone-Schoneberg. Dept. of Biotechnology Research, Pioneer Hi-Bred International, 7300 NW 62nd Ave., Johnston, IA 50131

Bombardment of tobacco and sunflower explants by tungsten microprojectiles prior to co-cultivation with *Agrobacterium tumefaciens* provided an improvement in stable transformation frequencies when compared to standard particle/plasmid or *Agrobacterium* methods. Tobacco leaves wounded by microprojectiles suspended in TE buffer prior to *Agrobacterium* treatment produced 100 fold more kanamycin-resistant colonies than leaves transformed by particle/plasmid bombardments. The identical construct designed to constitutively express NPTII was used in plasmid form for particle bombardments or within t-DNA of the binary *Agrobacterium* strain, EHA101. Experiments with sunflower meristems using plasmid or binary forms of a β-glucuronidase construct revealed that the frequency of transient GUS expression in meristems was high when bombarded by particles associated with plasmid. Such events did not lead to usable frequencies of stable transformation several week old plantlets. Large sectors of GUS expression in plants recovered from treated meristems were observed, however, only when the explants were first bombarded with particles in TE buffer before *Agrobacterium* treatment. Methods have been developed to recover transgenic plants and progeny from sunflower meristem explants treated by particle/*Agrobacterium* protocols.

P-10 Transformation of Recalcitrant Crops using Electric Discharge Particle Acceleration. PAUL CHRISTOU. Agracetus Inc., 8520 University Green, Middleton, WI 53562.

Application of recombinant DNA methodology to the genetic improvement of major agronomic crops is now a reality. During the last few years, we have witnessed tremendous advances in cell biology and gene transfer technology that enabled recovery of transgenic plants from even the most recalcitrant of crops. Electric discharge particle acceleration resulted in the development of variety-independent, commercial gene transfer processes for recalcitrant species. Using rice and soybean as examples to illustrate transformation of monocotyledonous and dicotyledonous species respectively, the versatility and advantages of this novel gene transfer method will be discussed.

P-11 Genetic Manipulation of *Agrobacterium tumefaciens* for the Transformation of Recalcitrant Plant Species S.B. GELVIN and C.-N. Liu Department of Biological Sciences, Purdue University, West Lafayette, In. 47907

We have tested various modifications of *Agrobacterium tumefaciens* for the purpose of increasing the transformation efficiency of various plant species. Transient transformation of plant cells was determined by staining for β-glucuronidase (GUS) activity four days following infection by *A. tumefaciens*. Binary vectors contained a *gusA* gene that does not express in the bacterium either because the Shine-Dalgarno ribosome binding site has been modified, or because of an intron within the gene. The T-DNA region of the vectors also contained a *nos-nptII* fusion gene and the T-DNA borders from either an octopine- or a nopaline-type Ti-plasmid. Some vectors also contained an additional copy of the *vir* gene transcriptional activator *virG*. *A. tumefaciens* strains harboring these plasmids contained either octopine-, nopaline-, or (supervirulent) agropine-type *vir* genes. Results of transformation of a number of different plant species indicate that some combinations of T-DNA borders and *vir* gene complements functioned better than others. In some plant species, the additional copies of *virG* harbored by the binary vector enhanced transformation. Finally, additional copies of *virG* relieve the acidic pH requirement for *vir* gene induction, permitting induction in medium of alkaline pH.

P-12 Marc Van Montagu
Laboratorium voor Genetica, Belgium

NO ABSTRACT SUBMITTED

P-13 Silicon Carbide Fiber-mediated Transformation of Plant Tissue Cultures. D.A. SOMERS and H.F. Kaeppler. Department of Agronomy and Plant Genetics, Plant Molecular Genetics Institute, University of Minnesota, St. Paul, MN 55113.

Development of alternative transformation strategies will provide researchers with increased flexibility in transforming plants. We have been investigating silicon carbide fiber-mediated DNA delivery into plant cells. Parameters affecting the efficiency of DNA delivery and stable transformation frequency have been investigated. Plant suspension culture cells were combined with silicon carbide fibers and plasmid DNA carrying the E. coli gene encoding β-glucuronidase (GUS). DNA delivery was mediated by either vortexing the treatment mixtures or agitating in a Wig-L-Bug agitator to effect fiber penetration of plant cell walls. Optimum DNA delivery procedures have been developed for regenerable and nonregenerable maize, oat and tobacco tissue cultures using transient GUS expression assays to determine DNA delivery frequencies. Beginning with a general set of DNA delivery parameters, it was rapid, simple and inexpensive to optimize DNA delivery for these species. Delivery of plasmid DNA carrying plant cell selectable markers has also been conducted in tissue cultures of the same species followed by selection of stable transformants. Stably transformed tissue cultures of nonregenerable maize and tobacco tissue cultures have been recovered. Southern blot analyses indicated integration of 1-20 copies of the transgene sequences into genomic DNA of the selected tissue cultures. Rearrangements of the integrated sequences were also observed and were similar to DNA rearrangements caused by other direct DNA delivery procedures. Selection of transformed, regenerable tissue cultures following silicon carbide fiber-mediated DNA delivery is also underway in an attempt to develop a plant transformation system based on this novel DNA delivery procedure.

P-14 On the Nature of Cell Heritable Variation in Culture. F. MEINS, JR. and M. Seldran, Friedrich Miescher Institute, Box 2543, CH-4002 Basel, Switzerland.

Plant cells in culture undergo extensive heritable variation. This variation is of considerable practical importance. When tissue culture is used for clonal propagation or as a system for genetic manipulation, untoward variation must be minimized. On the other hand, spontaneous mutations arising in culture provide novel forms of variation for use by the plant breeder. In principle, variants can arise by a *preadaptive* process in which new heritable cell types occur at random and are selected for by the culture procedure or by a *postadaptive* process in which the conditions of culture result in a directed change in heredity. The critical parameter used to distinguish between these two processes is the rate of variation *per* cell generation. Here, we outline a general method for measuring this parameter and apply the method to analyze variation in the requirement of culture tobacco leaf cells for cell-division promoting factors such as the cytokinins. Our results provide evidence for a novel type of pseudo-directed variation. Leaf cells "flip-flop" between a cytokinin-requiring and cytokinin-autotrophic phenotype at rates > 10^{-2} *per* cell generation. Although this heritable variation is preadaptive, because of its high rate, it appears to result in directed changes at the tissue level.

P-15 Implications of DNA Methylation Alterations. R.L. PHILLIPS and S.M. KAEPPLER. Department of Agronomy and Plant Genetics and Plant Molecular Genetics Institute, University of Minnesota, St. Paul, MN 55108

Plant tissue culture has become an important tool for genetic manipulation. Mutational events in the cultured cells can be exploited for various purposes but may be considered deleterious for other applications. Single gene mutations, transposable element activation, quantitative trait variation, and changes in chromosome structure and number occur in frequencies much higher in progenies of regenerated plants than controls. Given that the vast majority of such genetic changes go undetected, plant cells growing in the artificial culture environment must be making numerous genetic mistakes. Because such a variety of genetic alterations occur at elevated frequencies in culture, the question keeps arising as to how they can be causally related and whether there could be a common underlying cause. Using eighteen single-copy PstI genomic probes and two cDNA probes, we found extensive demethylation of sequences among regenerated families of maize. Unexpectedly, fifteen percent of the methylation changes were apparently homozygous in the original regenerated plant. The results indicate that epigenetic DNA methylation changes are common and that homologues may be coincidently altered. Chromatin structure might be altered with such global changes in methylation and this could lead to changes in gene expression across relatively large regions and thereby influence quantitatively inherited traits. Chromatin changes also could lead to delayed replication of heterochromatin and subsequent chromosome breakage. Methylation of specific sites could activate transposable elements or cause single gene mutations. If epigenetic variation in DNA methylation is the underlying cause of tissue culture-induced variation, the question of stability needs to be addressed. Preliminary data indicate stability in progeny from self pollination but not necessarily from crosses.

P-16 In Vitro Selection for Plant Improvement. J.M. WIDHOLM. Department of Agronomy University of Illinois, Urbana, IL, 61801

While the emphasis today is on transformation for plant improvement, there are some advantages to in vitro selection for accomplishing the same goal. Transformation is limited by the availability of the desired useful genes and presently transformed plants cannot be released into the environment. Selection for herbicide resistance has been accomplished with many species and varieties are about to be released for commercial use. Selection of resistance can expand the use of some of the newer, more environmentally safe herbicides which are used at very low rates. In the case of atrazine resistance, resistant weeds selected in the field all have impaired photosynthesis, but in vitro selection has produced resistant mutants which appear to have normal photosynthetic rates. We have selected carrot cells resistant to the herbicide glyphosate, which have increased levels of the target enzyme, enolpyruvylshikimate-3-phosphate synthase, due to gene amplication. The resistance is relatively stable in cultures, but whether it is stable at the whole plant level and through meiosis is not known. We have also been able to use culture filtrates of the fungus ***Septoria glycines*** to select regenerable soybean callus to produce plants with resistance to the disease caused by this pathogen. This accomplishment is important because resistance has not been identified before by screening thousands of accessions from the soybean germplasm collection. We have also used in vitro growth assays to rate the abilities of soybean genotypes to be resistant to iron deficiency which can cause chlorosis on high pH soils. In vitro assays are easier than field screening and have less variability as well.

P-17 Establishment of Cell Cultures of *Taxus brevifolia* (Pacific Yew) for Taxol Production.
D.M. GIBSON and R.E.B. Ketchum. USDA, ARS, NAA, Plant Protection Research Unit, U.S. Plant, Soil, and Nutrition Lab, Tower Road, Ithaca, NY 14853.

The bark of *Taxus brevifolia* (Pacific Yew) has provided the source for the novel chemotherapeutic drug, taxol. In ongoing clinical trials, taxol has shown great success in the treatment of ovarian cancer and looks promising for the treatment of breast, lung, colon, and other cancers. Supplies of the drug are limited because of the relatively rare, slow-growing, and uncultivated nature of the Pacific yew found in old growth forests of the Pacific Northwest. The complex chemical structure of taxol has eluded complete chemical synthesis, although partial synthesis using taxane precursors may be an alternate route. Cell cultures also offer an alternative, sustainable source for taxol production. Methods have been developed for generating callus and suspension cultures of *Taxus* sp. from bark, needles, stems, and embryos. Optimal growth for selected cell lines has been obtained on a B5 medium supplemented with casein hydrolysate and on a defined amino acid medium. Work to date has shown significant cell line variation for biomass and taxane production in reponse to light effects. Several major taxanes, including taxol, cephalomannine, and baccatin, have been extracted from cultures and analyzed via C18 and phenyl reverse phase HPLC. The largest source of variation for taxane production is dependent on cell line. Our current efforts are centered on selection of elite cell lines for taxol production.

P-18 Randy Strode
Agri Starts, Inc., USA

The History of the Micropropagation Business

NO ABSTRACT SUBMITTED

P-19 Current Micropropagation Production System,
R. D. HARTMAN, Twyford Plant Laboratories, Inc., 11850 Twitty Road, Sebring, Florida 33870

Commercial micropropagation laboratories describe their production systems using terminology originally defined by Murashige (1974) which includes Stages I-III. Because it takes between 9 to 60 months from the initiation of an individual plant variety to production, elaborate production control, build up and inventory control systems are required to effectively manage the micropropagation process profitably. Product quality and production reliability can be maintained by certification systems which are responsible for periodic infusions of clean, specific pathogen free, genetically true to type cultures, into the production process. The entire process of commercial micropropagation must to be managed using appropriate cost accounting and control mechanisms. Any number of labs have closed their doors because of being either under capitalized or failing to adequately account for their cost of operation and thus selling their product below costs. In a highly competitive global market profitability continues to be a major problem confronting commercial labs in industrialized countries. To date commercial micropropagation remains a labor intensive operation giving countries with low labor costs an advantage. Mechanization of certain steps, advanced production technologies, efficient lab designs, proximity to markets and proprietary varieties help mitigate these labor advantages.

P-20 Current Application of Micropropagation
G. R. HENNEN
Oglesby Plant Laboratories, Inc.
Route 2 Box 9, Altha, Florida 32421 USA

Commercial plant tissue culture has become a large and important segment of horticultural industries. The application of tissue culture propagation to a wide range of plants is the culmination of years of research by commercial and university laboratories. Today, tissue culture is used as a propagation tool in diverse horticultural areas. The possibility of year round delivery of plants allows for programmed production of food, ornamental and flowering crops. Tissue culture is an ideal tool for introducing new plant varieties by greatly reducing scale up times. In the United States, the vast majority of new plant releases originate from tissue culture companies or programs that utilize tissue culture as their release mechanism. Continued utilization of tissue culture technologies in the 1990's will further the development of improved plant varieties and cropping methods.

P-21 THE FUTURE OF THE MICROPROPAGATION BUSINESS

Irwin Chu, Twyford International Inc.

Currently, the micropropagation business is still limited to foliage, part of ornamentals and a small number of vegetables and other crops. Production cost and product stability are two major factors creating this limitation. Future technical development will concentrate on these areas and we anticipate that a great breakthrough will be made in the next ten years.

Various automation systems are being developed by many organizations worldwide, including: Disposable container formation, automatic media making, robotic plant cutting and transplanting. We estimate that the production costs will be reduced by more than half by the year 2000.

Stability of product quality, true-to-type and production control are still not satisfactory in many crops. We anticipate that major technical developments will soon be made and the application of tissue culture production systems will be greatly expanded beyond foliage and ornamentals. Currently, several vegetable, plantation fruit and forest trees are beginning to be produced through tissue culture. Practical examples will be discussed in the presentation.

P-22 Gene Expression in Wood Formation and Genetic Mapping in Loblolly Pine
D. O'Malley, R. Whetten, W. Bao, C. Loopstra, D. Grattapaglia, P. Wilcox, J. Chaparro, S. McCord, and R. R. SEDEROFF.

Pines are a group of higher plants that have great ecological and commercial value, yet little work has been done to develop molecular approaches for genetic engineering or to accelerate breeding. We have been identifying genes involved in the formation of the xylem cell wall because of the potential for directed genetic modification of wood properties. Genes involved in lignin biosynthesis and cell wall proteins in xylem have been cloned and an extensin like protein has been purified. Promoters from xylem specific genes have been isolated and tested in transient assays. In addition we have used RAPD markers to create a 9 cM genomic map of loblolly pine with 191 markers. New methods and theory for extending mapping with RAPD markers into breeding programs will be discussed.

P-23 The Matching of Biological and Physical Parameters is Crucial for the Stable Genetic Transformation of Conifers.
D. D. ELLIS, Department of Horticulture, University of Wisconsin, Madison, WI 53706

The regeneration of transformed plants of conifers has only recently been reported for two species, *Larix decidua* and *Picea glauca*. With both species, the developmental stage of the material used for gene transfer played a major role in the successful regeneration of transformed plants. With *Larix*, *Agrobacterium rhizogenes* was used to infect young hypocotyl segments and the formation of transformed shoots was dependent on the juvenility of the hypocotyl. In our work with *Picea glauca*, particle acceleration was used to transfer and express foreign DNA into various target tissues especially somatic embryos. In *Picea glauca*, the developmental stage of the somatic embryo exposed to particle acceleration was crucial for the induction of transformed embryogenic callus and the eventual differentiation of transformed plants. Studies of the transient and long-term expression of the marker gene, ß-glucuronidase, indicated the importance of not only the developmental stage of the explant, but also the post-gene transfer environments. Further, the use of various promoter constructs suggests that the expression of most introduced genes is suppressed very early after gene insertion. Our data provides insight into factors which are important for balancing the physical process of a gene transfer method with a biological system which is competent not only to express introduced genes, but is also competent to sustain gene expression over the time interval necessary for the regeneration of transformed plants.

P-24 Genome Mapping in *Populus*: Genetic Dissection of Adventitious Shoot Formation in vitro.
H.D. BRADSHAW, JR., K-H. Han, B.D. Watson, and R.F. Stettler*. Department of Biochemistry and *College of Forest Resources, University of Washington, Seattle, WA 98195.

The genetic improvement of trees for increased yields of lumber, fuel, and fiber is a primary goal of forestry in managed stands. Forest genetics is complicated by the long generation interval and outcrossing mating habit of most trees, and by the fact that most traits of biological and commercial interest are quantitatively inherited (polygenic). Quantitative traits may be dissected into their discrete Mendelian components using detailed genetic linkage maps in conjunction with two- or three-generation pedigrees. Once identified, such quantitative trait loci can be manipulated by combining traditional tree breeding with biotechnology to accelerate genetic gain. To identify genetic components of biomass productivity in a fast-growing hardwood, we are constructing a restriction fragment length polymorphism (RFLP) map in a *Populus* pedigree founded by a female *P. trichocarpa* and a male *P. deltoides*. The F_1 hybrids show striking heterosis for growth, and their F_2 and backcross offspring segregate for a wide variety of characteristics including growth, form, phenology, and disease resistance. When stem explants of the female *P. trichocarpa* parent are placed on Woody Plant Medium supplemented with 5uM trans-zeatin, adventitious shoot formation occurs with 100% frequency; in contrast, explants from the *P. deltoides* male parent fail to produce adventitious shoots. In the F_2 and backcross, continuous segregating variation from 0-100% is found. By correlating adventitious shoot formation in vitro with RFLP genotypes, we hope to elucidate the underlying genetics of organogenesis in a woody plant. This work should give further insights into the molecular mechanisms of trans-zeatin action, and may provide practical benefits for the regeneration of transgenic trees from genetically transformed cells.

P-25 Structure and Evolution of Photosynthesis Genes in Pines.

Petter Gustafsson, Jonas Lidholm, Stefan Jansson and Anna-Karin Lundberg, Department of Plant Physiology, Umeå University, S-901 87 Umeå, Sweden

The photosynthetic apparatus of gymnosperms is very similar to its angiosperm counterpart. However, many gymnosperms, including pines, produce chlorophyll, chlorophyll binding proteins and most if not all other photosynthetic proteins when germinated in the dark. Being evergreens, they are exposed to severe winter stress and they are evolutionary old; present day pines are believed to have evolved around 180 million years ago.

The chloroplast genome in pines, as well as in most other gymnosperms, lacks one of the inverted repeats making it different from most angiosperms. In addition, *Pinus contorta* and *banksiana*, but no other pines tested, carries a unique duplication of the *psbA* gene, coding for the reaction center II polypeptide D1. The duplicated psbA gene has been translocated to a position 2,4 kb upstream of the original copy. The duplication event seems to have taken place by homologous illegitimate recombination between 7 to 8 bp identical sequences. The duplicated *psbA* gene copy has been inserted between the promoter and 5' exon of the *trnK* gene resulting in a new fused *psbA-trnK* transcript indicating that the $tRNA^{Lys}$ is now expressed and regulated from the *psbA* gene promoter. On the chloroplast genome we found homologues to the *gidA* and *frxC* genes, genes that have been implicated in the production of chlorophyll in the dark in the green algae *Chlamydomonas reinhardtii*.

The chlorophyll *a/b*-binding proteins in the light antennae of photosystems I and II are coded for by gene families located in the nucleus. We have isolated and sequenced the pine counterparts to most of the genes that have been found in angiosperms and find that all chl *a/b* proteins are highly conserved implying very conserved functions for all these proteins. These genes were found to be expressed in dark-grown pine seedlings.

P-26 Effectiveness of Thidiazuron and CPPU as Cytokinin-like Compounds. P.E. READ, G. Yang and C.O. Auko. Dept. of Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68583-0724.

Traditionally, compounds considered as cytokinins have a purine-based structure (Skoog et al, 1965). However, Shantz and Steward (1955) identified a phenylurea compound as having cytokinin-like activity and later substituted ureas such as 4-pyridylureas were also found to possess cytokinin-like activity (Bruce and Zwar, 1966, Bruce, et al, 1965). In our research, both thidiazuron (N-phenyl-N'-1,2,3-thiadiazol-5-ylurea, "Dropp", TDZ) and CPPU [N-phenyl-N'-(2-chloro-4-pyridyl) urea] have stimulated unusually strong cytokinin-like responses in model systems such as petunia leaf segment culture (Fellman, et al, 1987). Both Thidiazuron and CPPU have also been found to elicit in vitro shoot formation in woody species much more effectively than would be achieved with traditional purine-based cytokinins. In azaleas, for example, the optimum CPPU concentration for shoot formation was one one-hundredth of the optimum for zeatin (0.5μM and 50μM, respectively). In recent studies using a forcing solution technique, TDZ and CPPU have been shown to apparently move in the transpiration stream, exerting their effects on softwood outgrowth of spirea and Acanthopanax. Per cent bud break was enhanced by TDZ in the forcing solution, but delayed the onset of bud break for both genera. In a recalcitrant species such as Castanea dentata, excellent in vitro shoot proliferation was stimulated by as little as 0.05μM CPPU or TDZ in the medium. Several additional woody species that have hitherto proven difficult to micropropagate have responded positively to CPPU, TDZ or both.

P-27 Effects of Paclobutrazol on the Development in vitro of Chrysanthemum, Rose and Grapevine.

A.V. ROBERTS[1], E. F. Smith[1], J. Mottley[1] and I. Gribaudo[2].

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[2] Centro Miglioramento Genetico Vite CNR, 10126 Torino, Italy.

Paclobutrazol is a triazole growth retardant which inhibits kaurene oxidase activity in gibberellin biosynthesis and 14 α-demethylation in sterol biosynthesis. In chrysanthemum, rose and grapevine cultured in vitro, it reduces fresh/dry weight ratios, shortens and thickens stems and roots, increases deposition of epicuticular wax and improves stomatal physiology. These changes are accompanied by an improved resistance of plants to desiccation which can also be demonstrated in response to some, but not all, of various other growth retardants which have been tested. There are likely to be applications of paclobutrazol-induced effects in various branches of plant tissue culture including micropropagation and adventitious regeneration.

P-28 The effects of thidiazuron and other cytokinins on plant regeneration and transformation in carnation and rose.

C. LU, G. Nugent, S. Tsuda and R. Young. Calgene Pacific Pty Ltd, 16 Gipps Street, Collingwood, Victoria 3066 Australia

Thidiazuron is reported to have cytokinin activity and has been shown to increase shoot regeneration frequency in a number of species. In developing regeneration systems for carnation, we have compared the effect of thidiazuron, 6-benzylaminopurine (BAP), kinetin and zeatin on shoot regeneration from petal and stem explants. Murashige and Skoog's (MS) medium was used as the basal medium. Thidiazuron was found to be most effective in stimulating adventitious shoot formation from carnation petal and stem explants. In rose, no shoot formation was observed on explants cultured on medium containing BAP or zeatin whereas adventitious shoots were induced on petal, receptacle and leaf when these explants were cultured on medium supplemented with thidiazuron.

Although thidiazuron is more effective than BAP in inducing shoot formation in carnation, no transgenic shoots were obtained when thidiazuron was included in the cocultivation medium. Transgenic carnation shoots could be recovered after cocultivating stem segments with Agrobacterium and culturing on MS medium with BAP. More transgenic shoots were produced when cocultivation was carried out with α-naphthaleneacetic acid or 2,4-dichlorophenoxyacetic acid. Comparison of β-glucuronidase (GUS) expression after cocultivating rose explants on MS medium with BAP or thidiazuron indicated that a lower concentration of thidiazuron than used for regeneration might be beneficial in obtaining transgenic shoots, as low levels of this growth regulator gave good transient GUS expression.

P-29 Kenneth C. Torres
Sigma Chemical Company

NO ABSTRACT SUBMITTED

P-31 Growth Regulation and Hardening of Bioreactor Regenerated Plants by Growth Retardants. MEIRA ZIV. The Hebrew University of Jerusalem, Department of Agricultural Botany and the Warburg Center for Biotechnology, Rehovot 76100, Israel.

Plant growth retardants which inhibit gibberrelin biosynthesis are used for plant growth regulation in vivo and recently have also been used in vitro. Widespread application of plant micropropagation is impeded by low proliferation rates and intensive manual handling. This can be overcome by scaling-up and automation. Scaling-up requires the use of liquid cultures, which often causes abnormal shoot and leaf morphology. Abnormal leaves with non-functioning mesophyll and stomata cannot survive transplanting. Shoot morphogenesis in liquid media was controlled by the use of ancymidol or paclobutrazol. These growth retardants reduced leaf and internode extension and stimulated axillary and adventitious buds on meristemoid clusters. In geophytes and potato the developing buds were induced by the growth retardants to form storage organs, starch accumulation and a higher dry weight compared to the control. Growth retardants given during hardening enhanced chlorophyll and anthocyanin synthesis as well as normal leaves with higher cuticular waxes and functioning stomata. The use of growth retardants in the proliferation stage and in the hardening stage provide a solution for scaled-up bioreactor micropropagation.

P-30 Silverthiosulphate, silvernitrate and carbendazim as alternative plant growth regulators in tissue culture systems. P.C. DEBERGH, G. De Coster, A. Agneessens, A. Jantasilp. University Gent, Coupure links 653, B-9000 GENT, Belgium

Carbendazim is the fungitoxic ingredient of different fungicides. In our experiments it was used as supplement to tissue culture media, to avoid the development of fungal contaminants. The product can be autoclaved without any loss of activity, and there is no degradation over a normal culture period of 32 days. With Cordyline terminalis (C.t.) and Prunus avium (P.a.) no phytotoxic activity was revealed up to 160 μg/ml. In C.t. stage II-cultures axillary branching was promoted, but shoot elongation was inhibited.

Silver nitrate and silverthiosulphate (STS), were added to stage I- and II-media, to study their anti-ethylene activity in micropagation of Fagus sylvatica, Laurus nobilis, Hevea brasiliensis and Laburnum anagyroïdes. Their effect was most pronounced after filter sterilization, and STS was always more effective than silver nitrate. For most plants STS had a positive effect in the initial stage, but continuous use was detrimental. Ethylene in the gasphase was measured.

P-32 Hierarchical Control and Hormonal Modulation of Gene Expression in Seed Development. M.A. BOGUE, J. VIVEKANANDA and T.L. THOMAS. Texas A&M University, College Station, Texas 77843

A 2.4 kb upstream region of a sunflower gene encoding the seed protein helianthinin confers rigorous developmental expression to a β-glucuronidase (GUS) reporter in transgenic tobacco seeds with no detectable GUS activity in non-embryonic tissues. Discrete elements of the helianthinin upstream regulatory ensemble (URE) confer novel regulatory patterns when analyzed outside the context of the complete sunflower regulatory complex; these include *cis* regulatory elements that confer ABA-responsive gene expression in vegetative tissues. In the full-length helianthinin URE, these elements only respond to ABA in the developing seed suggesting that the helianthinin gene contains additional regulatory elements that ensure hierarchical control in the developing seed. In contrast, a 1.5 kb 5' upstream region of the carrot *lea*-class gene *Dc*3 drives GUS expression in developing transgenic tobacco seeds and also is expressed in vegetative tissues in response to ABA and desiccation. The global organization of the sunflower helianthinin genes and the *Dc*3 gene appear quite similar. Current data suggests that embryonic specification is maintained by the combinatorial interaction of multiple *cis* regulatory elements; these elements are primarily located in the promoter proximal region of each gene although more distal elements are probably important in the regulation of helianthinin gene expression in developing seeds. Additional distal regions have a modulatory function, including response to fluctuations in ABA concentrations. We speculate that the structure of the helianthinin and *Dc*3 UREs may reflect a general pattern in highly expressed genes of domesticated plants.

P-33 A Gene Network Controlling Glutamine and Asparagine Biosynthesis in Plants. Gloria M. Coruzzi, Fong-Ying Tsai, Timothy Brears, and Gabrielle Tjaden. New York University, Dept. of Biology, 1009 Main Bldg., Washington Square, New York, NY 10003.

Our studies involve a molecular dissection of the factors which regulate the biosynthesis of amino acids in the glutamine/asparagine biosynthetic pathways in plants. Analysis of the multigene families encoding glutamine synthetase (GS) and asparagine synthetase (AS) reveals that individual members of these gene families are expressed in distinct cell types and are differentially regulated by environmental factors such as light and during development (for review see ref. 1). Light acts to activate the expression of the gene for chloroplast GS and represses the expression of AS genes. Cytosolic GS and AS genes are coordinately induced during germination when glutamine and asparagine are synthesized to mobilize nitrogen. We are currently analyzing the promoter elements of these genes by mutational analysis in transgenic plants to characterize cis-acting DNA elements of the promoters and the trancription factors which control gene expression. These studies should uncover the molecular mechanisms which coordinate the expression of genes in a common biosynthetic pathway in plants and have practical applications for improving nitrogen use in plants.

(1) McGrath & Coruzzi (1991) The Plant Journal 1, 275-280. This research is supported by NIH grant GM32877 and DOE grant DEFG0289ER14034.

P-34 Functional Analysis of Pollen-Expressed Genes. SHEILA MCCORMICK, Judy Yamaguchi, Lori Dircks and Keith Hamby. Plant Gene Expression Center, USDA/ARS-UC-Berkeley, 800 Buchanan St., Albany, CA 94710.

We want to understand the regulatory circuits that control differential gene expression in pollen, and we want to determine the roles that pollen proteins play during pollen maturation and germination. Towards this end, we have characterized several pollen-expressed genes (LAT52, LAT56 and LAT59) from tomato. We used both transient and stable expression assays to define cis-acting sequences of these promoters that are important for pollen expression. The LAT56 and LAT52 promoters share a common sequence element, as do the LAT56 and LAT59 promoters; these elements are important for quantitative promoter activity. We are examining protein interactions with these cis elements towards the isolation of the genes that encode the trans-acting factors. We are also screening *Arabidopsis* for mutants in genes encoding trans factors that interact with the LAT promoters. The LAT56 and LAT59 proteins show extensive sequence similarity to the ragweed allergen *Amb a I* and to a tomato pistil-expressed protein, and some similarity to pectate lyases, while the LAT52 protein shows some sequence similarity to proteinase inhibitors and to a maize pollen-expressed gene. We are using immunological and transgenic plant analyses to determine the localization and function of these proteins. We are sequencing analogs of these genes from several other plant species, in order to further determine conserved features of the promoters and coding regions.

P-35 Modulation of Maize Gene Expression Using Trans-Activators and Dominant Negative Inhibitors. B. BOWEN[1], B. Drummond[1], E. Unger[1], L. Tagliani[1], S. Maddock[1], F. Solan[1], L. Sims[1], B. Roth[1], R. Parsons[2], R. Schmidt[2], E. Grotewold[3], and T. Peterson[3]. 1) Pioneer Hi-Bred Int'l, Inc., P.O. Box 38, Johnston, IA 50131; 2) Univ. California, San Diego, CA 92093; 3) Cold Spring Harbor Lab, Cold Spring Harbor, NY 11724.

Anthocyanin biosynthetic genes in maize are coordinately regulated by two transcription factors, R and C. Following micro-projectile bombardment of maize suspension cultures, 35S::R and 35S::C expression can be assayed either by counting the number of cells which turn red or by measuring A1:: or Bz1::luciferase activity. Dominant negative mutant R (R-I) and/or C (C-I) constructs inhibit Bz1::luciferase up to 100-fold more effectively than antisense-R or antisense-C constructs. Trans-activation of A1::luciferase by 35S::P is similarly inhibited by R-I and C-I but 35S::GAL4-VP16 mediated trans-activation is not. The phenotypes of transgenic maize plants expressing R-I or C-I are currently being studied. Maize seeds which lack the opaque2 (O2) transcription factor are high in lysine because they do not express 22kD zeins (z22). Dominant negative inhibitors of O2 which inhibit trans-activation of z22::luciferase in endosperm-derived cells are also being tested in transgenic maize.

T-1
Integration of In Vitro Tests into Pharmaceutical Safety Assessment. **O.P. FLINT**, Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4755, Syracuse, NY 13221.

The objective of preclinical toxicity testing is to estimate possible human risk. This presentation will focus on the possibility, today and in the near future, of including the results of in vitro tests in this process of risk estimation. The following topics will be discussed:

1. Prediction of toxicity using in vitro methods: Measurement of phototoxicity using a human epithelial cell line will be described to illustrate the difficulties inherent in test validation and in using an in vitro method as a stand alone alternative to animal or human studies.
2. Quantitative structure-activity studies using in vitro methods: In vitro QSAR studies have been applied to the selection of new non toxic candidates for development. The example given will be an in vitro method for detecting reproductive toxins.
3. Determination of toxic mechanism using in vitro studies: In some cases, in vitro studies can be used in an attempt to establish the nature of a toxic mechanism. A study of cephalosporin nephrotoxicity will be given using an in vitro method.
4. Synthesis: The examples discussed in this presentation suggest ways in which in vitro tests might be used effectively as components of the risk evaluation process. A flow chart will be shown for risk evaluation in which in vitro studies will take a natural place alongside the in vivo..
5. Conclusion: Any test, in vitro or in vivo, must be shown to be relevant to our determination of human risk. Having established this validity in one case we do not necessarily expect the test to be equally valid for all future cases. Thus, our acceptance of in vitro studies as components of risk evaluation must be a slow and evolutionary process. The tests themselves will also evolve to better meet our needs.

T-2 **A COLLABORATIVE APPROACH TO THE EVALUATION OF ALTERNATIVES TO THE EYE IRRITATION TEST USING CHEMICAL INTERMEDIATES, DIANA M. GALER, Warner-Lambert Co., 201 Tabor Rd., Morris Plains, NJ. 07950**
Programs for the evaluation of in vitro alternatives to the eye irritation test have thus far focused on series of structurally or functionally related compounds. These studies have generally been used to correlate ranking of irritation in the alternative systems to ranking of irritation potential in vivo. It is not yet possible to use the available tests to classify individual compounds regardless of chemical class as to irritation potential. A consortium of 11 companies was formed to evaluate 7 alternative models. Specifically, the tests used were the Bovine Corneal Opacity/Permeability, the EYTEX™ test (Ropak Laboratories), Neutral Red Uptake Bioassay in Normal Human Epidermal Keratinocytes (Clonetics Corporation), MTT Assay using TESTSKIN Living Dermal Equivalent (Organogenesis Inc.), Microtox Bioassay (Microbics Corp.) and the Chorioallantoic Membrane Vascular Assay and a computer-based structure-activity-relationship model (TOPKAT, Health Designs, Inc.). Each company selected 1-6 compounds using specific criteria for in vivo irritation potential, pH and solubility. To assure technical consistency and evaluation, all tests were conducted at a single location (Merck Sharp and Dome Laboratories in France for the Bovine Corneal Opacity/Permeability test and Microbiological Associates, Inc. for the remaining tests) using coded samples. Compounds tested were synthetic intermediates isolated during the manufacture of pharmaceutical and chemical products. After breaking the code, the results were pooled and analyzed relative to physical properties and in vivo activity. Results indicate that while it may not be possible to use a single test to classify compounds as to irritation potential, some of the alternative test models may be useful in prioritizing and reducing the number of in vivo irritation tests conducted.

T-3 Evaluating Heart, Liver and Kidney Toxicity In Vitro with Precision-Cut Tissue Slices. C.E. RUEGG, T.M. Greenwalt, and P.M. Silber. In Vitro Technologies, Inc. 5202 Westland Blvd, Baltimore, MD 21227.

Precision-cut tissue slices maintained in vitro represent a model for studying organ-specific biochemical and pathophysiological mechanism of toxicity. The Krumdieck tissue slicer allows for the rapid production of slices of uniform thickness under physiological conditions. These slices exhibit normal tissue architecture and can be maintained for extended periods of time (24 hours) in suspension culture. In this study, slices prepared from heart, liver and kidney were used in vitro to evaluate chemical toxicity and target-organ sensitivities. A variety of alcohols, metal salts, pharmaceutical agents, and other toxicants were dosed onto these tissue slices for 22 hours, after which tissue-specific toxicity was measured by monitoring MTT absorption/conversion and histopathology. The results of this study indicate that slices from different tissues discriminate among tissue-selective toxicants. Specifically, the cardiotoxicant doxorubicin was far more potent in heart tissues than in kidney, whereas the mitochondrial electron transport inhibitor antimycin A was more potent in kidney than in liver, and the hepatotoxicant allyl alcohol was most toxic to liver. This model also demonstrates utility for rank-ordering the toxicity of a chemically related compounds. For example, the order of toxicity of alcohols in liver was allyl alcohol > methanol = ethanol, while in kidney slices cis-platinum was more toxic than its isomer trans-platinum, and metals rank-order as Cd > Zn ≥ Hg > Cr >> Ni >> Mg. This study demonstrates that an in vitro tissue slice battery can provide a useful tool for product development and safety assessment by rapidly identifying target organ susceptibility, chemical potency, and (through the evaluation of multiple endpoints) mechanism of action of a wide variety of chemicals.

T-4 Understanding Secondary Mechanisms of Carcinogenicity Using In Vitro Approaches. J. M. Lipman, Hoffmann-La Roche Inc., Nutley, NJ 07110-1199

The Delaney Clause of the FD&C act prohibits the use of carcinogenic food and color additives as well as animals drugs used in food production. Under this clause Sulfamethazine and other sulfonimides would be removed from the animal health market. Currently, it is believed that these and other compounds work by mechanisms other than direct carcinogenic action. The thyroid gland tumor response to sulfonimides in rodents is considered to be secondary to hormone imbalance at high doses and not the result of a direct carcinogenic effect (conceptually referred to as a secondary mechanism of carcinogenesis). Most research in this area has been conducted in animals, with in vitro techniques being applied to help elucidate these mechanisms. We will review both the current status of in vivo and in vitro studies demonstrating the importance of both approaches in understanding mechanistic questions of regulatory importance.

T-5 Marie Chow
Massachusetts Institute of Technology, USA

Overview of Antiviral Strategies

NO ABSTRACT SUBMITTED

T-7 Jeff Gordon
Washington University, USA

Protein Acylation: Potential Targets for New Antiviral Therapies

NO ABSTRACT SUBMITTED

T-6 Molecular mechanisms of Action of Ribavirin - Ribavirin (1-B-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) or virazole is a broad-spectrum antiviral agent whose molecular mode of action remains remarkably controversial. The drug was approved by the Food and Drug Administration in 1986 for aerosol use in infants with serious infections due to respiratory syncytial virus (RS). Ribavirin is and has been under clinical investigation against a variety of viral illness, including those due to influenza virus, Lassa fever, Korcan hemorrhagic fever with renal syndrome (KHFS) and human immunodeficiency virus (HIV). The drug possesses inhibitory activity against a broad spectrum of viral pathogens, including both DNA and RNA viruses, intrinsically suggesting a vast clinical potential that has yet to be realized.

There has been a great deal of clinical interest in utilizing ribavirin for HIV infections. It has been reported to slow the development of AIDS in HIV infected patients, The mechanism for the interference of ribavirin on HIV is as yet not understood. Recently Vogt et al (3) reported that this drug was antagonistic with AZT in HIV infected cells. They hypothesized that since both drugs work in some part as a triphosphate that there is competition for cellular kinases.

Several theories regarding the molecular mode of action of ribavirin have been proposed. One hypothesis states that the drug leads to decreased intracellular pools of GTP, indirectly suppressing viral nucleic acid synthesis. Another hypothesis proposes that ribavirin therapy of viral-infected cells results in the synthesis of RNA with abnormal or absent 5' cap structures, which in turn leads to inefficient translation of viral transcripts. A third hypothesis states that the drug has a direct suppressive effect on viral polymerase activities. Experimentally it has been difficult to determine the primary mechanism of action because none of these hypothesis are mutually exclusive, and indeed, they may indicate that ribavirin acts in a complex, multiple site fashion. It is also important to note that ribavirin, unlike all other drugs whose structures resemble nucleoside analogues, has a modified base.

T-8 Use of Structure in the Design of Anti-viral drugs: Viruses require a number of activities which are unique to the virus and are therefore, suitable targets for intervention with antiviral agents. In traditional methods of drug design, lead compounds are identified by screening a large number of chemicals for a desired activity, and the activity of the lead is improved via structure activity studies of the pharmacophore. These methods are responsible for the development of currently used antivirals. Thus, successive design utilizing nucleoside analogues led to the development of acyclovir (a potent inhibitor of the replicase in Herpes simplex and Herpes zoster) and to the development of AZT (a potent inhibitor of the reverse transcriptase of HIV). Rapid advances in structural methods for macromolecules have begun to expand these programs to include parallel studies of the macromolecular targets of the drug as well. For example, the recent elucidation of the structure of the acid protease of HIV, has contributed significantly to the design of small molecules which inhibit the protease at nanomolar concentrations. In the future, use of the structure of the target as well as the pharmacophore are expected to play an increasingly important role in the development of antiviral agents.

Even simple viruses, such as the picornaviruses (which include polioviruses, rhinoviruses, foot-and-mouth disease virus, and hepatitis A virus) provide a number of targets for antiviral intervention. We have initiated a program which include crystallographic studies of viral enzymes, including the viral RNA-dependent RNA polymerase and viral proteases, and complexes of virions with drugs which bind virions and interfere with structural transitions associated with cell entry. The studies of complexes with capsid-binding drugs will be used to demonstrate possible approaches to the use of macromolecular target structures in "rational" antiviral drug design.

T-9 Cooperation of Two Cell Types for the Assembly of sIgA in Culture

J.P. KRAEHENBUHL, R. Hirt, and N. Jeanguenat, Swiss Institute for Experimental Cancer Research and Institute of Biochemistry, University of Lausanne, CH-1066 Epalinges

Two cell types, mucosal plasma cells and epithelial cells cooperate to produce secretory IgA antibodies that protect mucosal surfaces against environmental pathogens. Secretory IgA consists of IgA molecules oligomerized via J chain and associated to secretory component (SC) which corresponds to the five Ig-like domains of the polymeric Ig (poly-Ig) receptor. The receptor, expressed in epithelial cells of the digestive, respiratory and genital tracts, mediates transepithelial transport of IgA antibodies. It is cleaved during transport or at the lumenal surface and free or bound SC is released into the lumenal medium. Association of SC to IgA renders the complex resistant to proteolysis. We have reconstituted *in vitro* such a system by co-cultivating IgA-producing hybridoma cells and poly-Ig receptor-expressing epithelial cells. MDCK cells, transfected with rabbit poly-Ig receptor cDNA inserted into a glucocorticoid-inducible expression vector, were grown to confluency on permeable supports (Transwell filters) while hybridoma cells were embedded in the lower chamber of the Transwell device in a collagen gel to prevent cell proliferation. Secretory IgA antibodies are recovered exclusively in the upper medium, indicating that transport is vectorial. The receptor's itinerary and transcytosis has been shown to depend on the phosphorylation of two serine residues on the cytoplasmic tail of the receptor.

T-10 Cultured Brain Microvessel Endothelial Cells: An In Vitro Model of the Blood Brain Barrier. RONALD T. BORCHARDT. Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66045.

The mammalian blood-brain barrier (BBB) is formed by a continuous layer of brain microvessel endothelial cells (BMEC's) which are characterized by highly resistant tight intercellular junctions, minimal pinocytotic activity and the absence of fenestra. Along with these features of an active anatomical barrier, the BMEC's also pose a formidable enzymatic barrier involved in the metabolism of a broad range of blood-borne substrates. In addition to restricting the passage of most small, polar molecules (e.g., peptides) into the brain, the BBB also effectively limits the movement of most macromolecules (i.e., proteins) between the circulation and the brain interstitium. It is recognized, however, that the BBB is selectively permeable to some endogenous small polar molecules and macromolecules. Thus, an understanding of the physiological mechanisms by which these endogenous molecules are able to gain access to the brain is essential to developing rational strategies for targeting drugs to the brain. To aid in the elucidation of these mechanisms and to provide a simple, rapid and inexpensive model to test strategies designed to circumvent the BBB, our laboratory developed an in vitro model of the BBB consisting of primary cultures of bovine BMEC's grown onto plastic surfaces or microporous polycarbonate membranes. These cultured bovine BMEC's have been shown to retain many of the anatomical, biochemical and transport characteristics of the BBB in vivo (Y. Takakura, K. L. Audus and R. T. Borchardt, Advances in Pharmacology, 22, 137-165, 1991; K. L. Audus and R. T. Borchardt, Handbook of Experimental Pharmacology, R. L. Juliano, Ed., Springer, Berlin, p. 43-70, 1991). This presentation will include a description of the methodology used to isolate and culture BMEC's and a summary of the results of peptide and protein transport experiments.

T-11 Drug and Peptide Absorption in Human Intestinal Epithelial Cell Cultures. P.ARTURSSON, Dept.Pharmaceutics, BMC, Box 580, Uppsala University, S-751 23 Uppsala, Sweden.

Monolayers of well differentiated human intestinal epithelial cell lines grown on permeable filters, have been used to quantify different barriers to drug and peptide absorption. For this purpose, cell lines representing absorptive as well as goblet cells have been characterized. The results indicate that extracellular barriers such as the aqueous boundary layer and the mucus layer are of limited importance in restricting drug and peptide absorption. Cellular barriers, including the cell membranes and the tight junctions are more important. Therefore, methods for the determination of unbiased cell permeability coefficients have been developed. Monolayers of the absorptive cell line Caco-2, which have a permeability comparable to human colon, have been used to predict the in vivo absorption of structurally different drugs and peptides. Caco-2 monolayers have also been used to study the poorly understood effects of pharmaceutical additives and various drug delivery systems on the intestinal epithelium. More specifically, compounds and drug delivery systems that increase the absorption of drugs and peptides, so called absorption enhancers, have been studied. The results indicate that many absorption enhancers have an unselective mechanism of action which involves wounding of the apical cell membrane, redistribution of actin filaments and separation of tight junctions. Drug delivery systems that selectively and reversibly enhance drug and peptide absorption across the tight junctions have been found and their mechanisms of action are currently under investigation.

T-12 The Transport of Vitamin B_{12} Across Monolayers of Caco–2 Cells Grown on Permeable Supports

I F HASSAN and M Mackay;
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Caco-2 cells grown on permeable supports form intact monolayers with many of the properties of polarised intestinal epithelial cells. A unique characteristic of distal ileal epithelial cells is the receptor-mediated endocytic transport of cobalamin (Cbl, vitamin B_{12}). We have demonstrated that Caco-2 cells will bind and internalise intrinsic factor-Cbl complexes and after 14-28 days in culture this specific binding is exclusively located on the apical membrane. Specific transcellular transport of [^{57}Co]-Cbl apical to basolateral, occurs between 20-28 days in culture. In addition, Caco-2 cells synthesise and secrete a protein, transcobalamin II (TCII), which is believed to be responsible for the transport of Cbl through intestinal epithelial cells. After 20 days in culture, this protein is secreted predominately from the basolateral side of Caco-2 cells. We have localised TCII to specific intracellular vesicles using immunocytochemical techniques and these vesicles have been fractionated on ficoll gradients. We believe that Cbl is released from intrinsic factor in a intracellular compartment, transferred to TCII and is secreted at the basolateral surface. Thus, Caco-2 cells provide the first polarised human cell system for studying the transepithelial transport of cobalamin. The suitability of this pathway to transport drug conjugates will be discussed.

T-13 Cultured Rabbit Tracheal Epithelial Cells: An *In Vitro* Model of Upper Respiratory Drug Penetration, C. S. SCHASTEEN, S.R. Rapp and B.T. Keller, Monsanto Company, St. Louis, Missouri 63167

The study of upper respiratory airway drug penetration and metabolism represents a challenge to the pharmaceutical scientist. A cell culture system for primary rabbit tracheal epithelial (RTE) cells growing on collagen-coated, transparent filter inserts has been developed as a model for studying drug penetration across the upper respiratory epithelium. The cells are isolated following protease dissociation from excised rabbit trachea and cultured in a defined growth medium. Using anti-epithelial keratin antibodies and immunofluorescence we have found that all of the viable cells are of epithelial origin. After reaching confluency within 3-4 days, we determined that epidermal growth factor must be omitted from the growth medium to prevent continued proliferation of the cells and to promote cellular differentiation. The onset of differentiation is marked by the formation of tight intercellular junctions between neighboring cells on the apical surface, a corresponding increase in transepithelial electrical resistance (TEER) to ~1200 Ω-cm^2 (i.e., a tight epithelial barrier), and a concomitant pH partitioning between the apical and basal chambers indicative of specific ion transport systems known to be present in tracheal epithelial cells. The cultured cells do not form a true, pseudostratified columnar epithelia as observed *in vivo*, but instead exhibit a more cuboidal morphology. At later times in culture (7-10 days), many cells (20-40%) become ciliated on their apical surface and these cells take on more of a columnar morphology. In addition to tight intercellular junctions and the presence of ciliated cells, the primary cultures also have distinctly large nuclei, numerous mitochondria, occasional mucin granules and mucin on the apical surface (histochemical staining) and a complex network of intermediate filaments, all of which are characteristic of the cells *in vivo*. Mucin synthesis is dramatically enhanced by culturing the cells without medium on their apical surface (i.e., air-interface). Penetration of 3 representative compounds across the RTE monolayers (apical to basal) showed a similar rank order to that reported for nasal bioavailability of the compounds *in vivo* (propranolol > enkephalin > thyrotropin releasing hormone). These results suggest that the RTE cells in primary culture retain many characteristics of the differentiated, intact upper respiratory epithelium *in vivo*, and thus, appear to be a useful model system for the study of drug penetration (and potentially metabolism) across this mucosal barrier.

T-14 Induction Of Heat Shock Proteins (HSPs) In Sertoli Cells By Cadmium. M.J. BRABEC, L.Batarseh and M.J. Welsh*. Chemistry Department, Eastern Michigan University, Ypsilanti, MI 48197 *Cell Biology and Anatomy, The University of Michigan, Ann Arbor, MI 48109

HSPs are a small group of highly conserved proteins that were first demonstrated to be induced in eukaryotic cells by exposure to hyperthermic conditions. The induction of the HSPs has since been shown to be associated with cellular responses to a wide range of chemical and physical stresses. Spermatogenesis, in addition to being heat-labile, is also sensitive to a number of chemicals, such as cadmium and lead. We are studying the role of HSPs in the response of primary cultures of Sertoli cells (SCC), isolated from rat testes, to a number of chemical agents. The phosphorylation of hsp27, revealed by 2D gel electrophoresis, is increased in cells exposed for 6 hrs. to 1 μM cadmium chloride and 1 hr to 100 μM lead acetate. The increased phosphorylation of hsp27 by cadmium is inhibited when either cycloheximide or actinomycin D are present, suggesting that the increase in phosphorylation is dependent upon protein synthesis. Determination of mRNA for hsp27 and hsp70 in SCC exposed to cadmium chloride by the polymerase chain reaction suggest that the increase of hsp27 phosphorylation can be at least partially attributed to an increase in hsp27 mRNA, and, presumably, hsp27 synthesis. These data suggest that the heat shock response is important in the response of the testes to inhibition of spermatogenesis by toxic metals. Supported by NIEHS RO1ES05298 and EMU Research Excellence Fund.

T-15 Molecular Interactions of Lead in Cultured Astroglia. E. TIFFANY-CASTIGLIONI. Dept. Veterinary Anatomy & Public Health, Texas A&M Univ., College Station, TX 77840.

Biochemical evidence concerning low-level (< 1 μM) lead (Pb) effects on cells supports two concepts: first, that Pb-induced damage is a continuous, culmulative process that begins at discrete molecular sites and progresses unless contained by defensive mechanisms within the cell; and second, that Pb insinuates itself into metabolic pathways normally used by essential metals. A model for astroglial damage by low-level Pb exposure that incorporates these concepts will be discussed. The model depicts stages through which a Pb-exposed cell passes, beginning with non-exposure and ending with cell death or pervasive cell dysfunction. Between these endpoints lies a continuum of metabolic changes consisting of initial injuries that take place at discrete molecular sites, followed by the metabolic amplification of such injuries. The toxic effects of lead become more telling until cells exhibit significant biochemical impairments with ultimate effects upon brain function. During all stages, the cells may develop compensatory mechanisms for Pb accumulation, such as the activation of Pb-binding proteins that permit temporary or long-term storage. Resistance to Pb toxicity in brain cells probably depends on a rather limited capacity to replace damaged molecular components. Thus far, we know very little about such mechanisms. One may predict that turnover of components must occur early in the toxic process so as to compensate for immediate damage that may have occurred. A new genre of cell culture assays is available that are based upon the quantitation of fluorescent probes in intact, living cells by interactive laser cytometry. These assays have great potential to be more powerful and toxicologically relevant than conventional in vitro assays and are also adaptable to automation. Laser cytometry data from this laboratory will be discussed within the framework of the above model.

T-16 Use of Isolated Hepatocytes and Organ Slices to Study in Vitro the Metabolic Injury by Metals.
F. Goethals, P. Buc-Calderon and M. Roberfroid, Unité de Biochimie Toxicologique et Cancérologique, Université Catholique de Louvain, BCTC 7369, Av. Mounier 73, B-1200 Brussels, Belgium

During the last two decades, in vitro models have been developed in order to support toxicological investigations. Among them, short term suspensions or long term cultures of rat hepatocytes are widely used. More recently, mainly because of new technological development, organ slices have regained much interest as a complementary model with the major advantage of retaining tissular organization and cellular interactions.

Our laboratory has been one of first to advocate the importance of metabolic parameters as early and sensitive indicators of cell injury by chemicals. Since, among the toxic chemicals, metals play an important role, part of our investigation was on metabolic injury by inorganic salts. As examples of such in vitro studies, we will report on the acute effects of $NiCl_2$ and Fe^{3+} plus hydroperoxides on various metabolic competences of isolated hepatocytes surviving in suspension. The use of tissue slices will be illustrated by reporting on the metabolic effects of sodium chromate and mercuric chloride on renal slices as a tool to investigate their nephrotoxicity.

T-17 In Vitro Modulation of human lymphocytes and monocytes by metals. D. A. LAWRENCE and J. M. Slavik. Departments of Microbiology, Immunology and Molecular Genetics and Pathology and Lab Medicine, Albany Medical College, Albany, NY 12208.
Numerous metals have been reported to be essential trace elements for lymphocyte activation and growth, and these metals as well as others also have been reported to be toxic at higher concentrations. This presentation evaluates the in vitro influences of the chloride salts of cadmium (Cd), copper (Cu), gold (Au), lead (PB), mercury (Hg), nickel (Ni), and zinc (Zn). The cytotoxicity of the metals was examined under low (5%) and normal (19%) percentages of oxygen by propidium iodide uptake assessed by flow cytometry. In general, the cytotoxicity of a metal correlated with its inhibitory activity; however, some metals such as Cd were much more inhibitory than cytotoxic. Furthermore, the metals usually were less cytotoxic at 5% oxygen. Interestingly, 2-mercaptoethanol (50 μM) enhanced the cytotoxicity of Hg (5, 10 and 50 μM) but had no influence on that of Cd, Ni, Pb and Zn. After in vitro exposure to 0 to 32 μM doses of metal, mononuclear cell preparations of lymphocytes and monocytes were assessed for mitogen-induced proliferation and immunoglobulin or cytokine production. Although Hg was the most cytotoxic metal, Cd was, overall, the most inhibitory. At select doses only three metals (Hg, Pb and Cu) significantly enhanced a particular response. Hg (4-8 μM) enhanced concanavalin A (Con A)-induced proliferation and IL-1 production. At 4-8 μM, Cu enhanced IgM production, and at 4-16 μM, Pb enhanced B cell proliferation and IgM production. In general, the Con A-induced response was the most sensitive of the mitogen-induced responses. Unlike IL-2 production which was inhibited by all metals, IL-1 production was inhibited only by Hg and Cd. Some metals were assessed for their ability to alter cell phenotype based on surface antigens. Hg appears to inhibit CD4 expression on monocytes but not lymphocytes and enhance CD23 expression on lymphocytes. Supported by Public Health Service Grant ES03179.

T-18 Tracheal Explant Cultures as Models for Lung Carcinogenesis. R.A. COULOMBE, Jr. Programs in Toxicology and Molecular Biology, Department of Veterinary Science, Utah State University, Logan, UT 84322-4620.
The cultured tracheal explant system is ideally suited for studies of carcinogen action and of the role of airway cells in the carcinogenic process. Cultured tracheas allow observations of histopathological changes within the context of intact tissue, and do not need serum or complex growth factors. Our laboratory has utilized tracheal explant cultures, derived from various species, to study the effect of the carcinogenic mycotoxin aflatoxin B_1 (AFB_1) in airway epithelium. Explants were derived from species whose tracheas contain an abundance (rabbit and hamster) or a scarcity (rat and monkey) of smooth endoplasmic reticulum (SER) in their non-ciliated tracheal epithelial (NC) cells. In mammalian upper airways, the majority of cytochrome P-450 is contained within SER-containing NC cells. In our studies, carcinogen action in airway cultures qualitatively related to airway morphology. For example, in cultures from the rabbit and hamster, AFB_1 was efficiently metabolized and activated to intermediates which form DNA adducts, and was selectively toxic to NC cells. Tracheal explants from rat and monkey were deficient in AFB_1 metabolism and activation, showed few signs of cytotoxicity. Repair of AFB_1-DNA adducts occurred at different rates in these cultures. Selective cytotoxic effects observed in hamster cultures were predictive of those seen following in vivo pulmonary administration of AFB_1 in the same species as part of a long-term study (Supported by NIH grant ES04813).

T-19 **Large Airway Cell and Organ Culture Systems for Exposure to Oxidant Gasses** D W. WILSON, R. Wu and B. Tarkington. School of Veterinary Medicine, University of California-Davis Davis, CA 95616
Documented large airway responses to inhalation of oxidant gasses include ciliary loss, selective necrosis of ciliated cells, secretory cell hyperplasia, and alteration in secretion and composition of glycoprotein in mucus cells. The mechanisms of cell injury and potential physiologic and inflammatory cell interactions underlying the epithelial cell response are difficult to elucidate without using in vitro systems. Such systems are complicated by the need to control and monitor the generation, delivery, and dosimetry of reactive gasses in an enclosed environment. Successful systems involve closed chambers with high flow of humidified gasses and either moving platforms or elevated membranes and basilar feeding systems to allow direct interaction of oxidants with epithelial cells. Exposures are done with either intact explants of airways or mixed epithelial cell cultures. Characterization strategies for epithelial cell cultures include glycoprotein secretion, cytokeratin expression, and ultrastructural demonstration of cellular polarity and apical junctional complexes. Tracheal explant preparations are frequently used in exposure systems because cultured airway cells lose important differentiation characteristics, particularly cilia, in the absence of a natural substrate. We have used both explant and epithelial cell cultures to study epithelial cell injury and adaptation to ozone exposure. Our experiments have demonstrated that 1) One to four day exposures of airway explants to ozone results in ciliated cell injury that is morphologically similar to that seen in vivo. 2) Adaptation to long term exposures in vivo is reflected in airway cells that are resistant to direct exposure in vitro. 3) Ozone and oxygen have different and synergistic effects on the airway epithelium. and 4) Established cell line cultures are more sensitive to ozone than primary cell isolates and this difference may be related to the amount of glycocalyx on the cell surface.

T-20 Isolation and Culture of Small Airway Cells for Experiments in Selective Toxicity and Metabolism. C. PLOPPER, C. Chichester, L. Stewart, A. Pang, A. Chang, W. Cardoso, R. Philpot and A. Buckpitt. School of Veterinary Medicine, University of California, Davis, CA 95616.
The targets for many bio-activated pulmonary cytotoxicants are the nonciliated bronchiolar epithelial (or Clara) cells of the small, distal airways. Two approaches have been used to define the relationship between metabolism and cytotoxicity: isolation of Clara cells from whole lung and isolation of bronchiolar airways by microdissection. The first involves a combination of enzyme digestion and centrifugation. Major considerations are that these procedures can produce significant proteolysis of relevant enzyme systems and that the isolate includes Clara cells from throughout the airway tree. Strategies are available to eliminate proteolysis of the cytochrome P-450 system and produce isolates of high purity. These isolates exhibit a dose- and time-dependant cytotoxicity with a known Clara cell cytotoxicant, naphthalene, and characteristic metabolic profile for P-450 metabolism of the compound. Bronchioles isolated by microdissection contain the Clara cells characteristic of bronchioles, which have a different sensitivity to naphthalene injury than do Clara cells in more proximal airways. However, they also contain other cell types (ciliated cells, smooth muscle cells and fibroblasts) in higher proportions than are present in isolates of Clara cells. By maintaining rigorous conditions during microdissection, enzyme activity for cytochrome P-450s and glutathione S-tranferase and glutathione levels are maintained. The explants show dose- and time-dependant cytotoxicity and a close relationship with metabolism. Isolated bronchioles can be maintained in culture for up to 30 days.
Supported in part by NIH grants ES04311, ES00628 and HL42032.

T-21 In Vitro Hamster/Human Clara Cell Differentiation for Lung Toxicology.
M. EMURA[1], A. Ochiai[1], M. Riebe-Imre[1], G. Singh[2], S.L. Katyal[2], J. Knebel[1], U. Mohr[1], D.L. Dungworth[3], J. Jacob[4] and G. Grimmer[4]. [1]Med. Hochschule Hannover, 3000 Hannover 61, FRG; [2]Department of Veterans Affairs, Pittsburgh, PA 15240, USA; [3]University of California, Davis, CA 95616, USA; [4]Biochemisches Institut für Umwelt-carcinogene, 2070 Grosshansdorf, FRG.

We previously described an undifferentiated epithelial cell line from the fetal Syrian hamster/human lung, which underwent differentiation with mucus-like granules under a specific condition (In Vitro Cell Dev Biol 24:639,1988; Virchows Arch 61:217,1991). We have later identified resemblance of the granules to those of Clara cells as well as a Clara antigen on the thus differentiated cells, which we now regard as an in vitro counterpart of Clara cells. This in vitro differentiation apparently proceeds through a precursor stage reminiscent of an in vivo "small mucus granule cell" (SMGC), a target of various carcinogens. Since an in vitro model for lung toxicity requires identified target cells, we have explored the applicability of the above system with more emphasis on the in vitro counterpart of SMGC. In vitro hamster/human SMGC metabolized 88/24% of chrysene (CHR), 97/60% of benzo(a)pyrene (BaP) and 30/25% of pyrene (PYR) in 3-8 days. The metabolites, 1,2-diolCHR, 3,4-diolCHR, 9,10-diolBaP, 7,8-diolBaP and 1-hydroxyPYR were 6/3, 12/12, 28/5, 3/~0 and 12/12%. Some other PAHs were also metabolized more quickly by hamster than human cells. In vitro hamster Clara cells were twice as active as in vitro hamster SMGC in metabolizing BaP in a 2-day incubation.

T-22 Timothy D. Phillips
Texas A&M University, USA

Use of Hydra and Rodent Embryo Cultures for the Study of Teratogenic Mycotoxins

NO ABSTRACT SUBMITTED

T-23 Development and Application of Mouse Blastocyst Culture Systems to Evaluate the Prenatal Toxicity of Mycotoxins. T. R. IRVIN, Institute for Environmental Studies, Louisiana State University, Baton Rouge, LA 70803

Repeated episodes of prenatal toxicity and mortality in both humans and animal populations chronically exposed to mycotoxins have focussed attention on these compounds as potential etiological factors in undiagnosed prenatal disease and birth defects. Our group has developed an in vitro cell culture system that permits characterization of the prenatal toxicity of individual mycotoxins as well as evaluation of the interactive prenatal and developmental effects of mycotoxin mixtures. This cell culture system, the primary culture of preimplantation mouse embryos on day 3 of gestation, allows direct observation of mycotoxin-induced effects on early embryonic development including trophoblastic hatching and embryonic implantation. Coincubation of enzyme fractions with this cell culture system has further allowed an assessment of the role of exogenous biotransformation on the prenatal effects of mycotoxins. Results will be presented characterizing, in qualitative and quantitative terms, the prenatal toxicity of aflatoxin B_1, the etiology of biotransformation of this compound in the developing mouse embryo, as well as the prenatal clastogenicity of aflatoxin B_1. Comparative data on various members of the aflatoxin family of toxins will be presented and compared to data in whole animals.

T-24 Action of Aflatoxin B_1 in Lung Airway Cultures. R.A. COULOMBE, Jr. Programs in Toxicology and Molecular Biology, Department of Veterinary Science, Utah State University, Logan, UT 84322-4620.

While the major research emphasis on the effects of aflatoxin B_1 (AFB_1) has correctly focused on hepatocarcinogenic effects from dietary exposure, people are also exposed to AFB_1-contaminated, respirable particles of grain dusts generated from the harvest, transport and processing of grains. Besides the hepatic effects from this exposure, it is also possible that AFB_1 poses an occupational lung cancer risk to workers who inhale these dusts. To explore this possibility, we have utilized intact cultured trachea from a variety of mammalian species to study the metabolism and action of this mycotoxin in lung tissue. Since mammalian airways are populated by a variety of cell types, the use of intact short-term tracheal cultures enables a determination of comparative susceptibility as well as of the cells involved in activating this mycotoxin. Our studies have shown that the smooth endoplasmic reticulum-containing nonciliated tracheal epithelial cells (NC) is the cell type that activates AFB_1 and is most susceptible to the cytotoxic effects of this mycotoxin. In tracheal cultures derived from species whose airways contain an abundance of NC cells (such as rabbit and hamster), AFB_1 is activated to DNA adducts in a pattern qualitatively similar to that seen in hepatic systems. In addition, AFB_1 is selectively absorbed and is selectively cytotoxic to NC cells in these cultures. Aflatoxin B_1 is neither appreciably activated nor is cytotoxic in tracheal cultures from species with few NC cells. (Supported in part by NIH ES04813).

T-25 Valery Smirnov
Kiev State University, Russia

Cell and Tissue Culture Evaluation of Mycotoxins Operative in Human and Animal Disease

NO ABSTRACT SUBMITTED

T-26 Primary Embryo Cell Cultures: Utility in Developmental Toxicity Studies
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This talk will discuss the utility of primary embryo cell cultures as methods for evaluating developmental toxicity. The method that will be highlighted is micromass cell cultures, high density cultures utilizing rodent or avian tissues. Either limb or cephalic tissues (usually mesencephalic regions) are isolated from early to mid-organogenesis embryos and single cell suspensions are prepared using a combination of mechanical dissociation and enzymatic digestion. Cells are plated at high density (usually 2 x 10^7 for limb cultures and 5 x 10^6 for CNS cultures). Cells undergo differentiation into chondrocytes or neurons without additional stimulation. Applications of these cultures to developmental toxicity evaluation have included structure-activity analyses, mechanistic research and screening. The advantages and disadvantages of these culture systems for each of these applications will be discussed using specific examples. Validation issues for these applications include legitimacy of endpoints for in vivo response prediction and sufficiency of in vivo versus in vitro comparison testing. Additional validation issues will focus on the feasibility of predicting both qualitatively as well as quantitatively an in vivo response using such in vitro information. The final portion of this presentation will present information describing future research directions required for full utilization of these culture techniques.

These studies were supported by NIH ES-03157 and ES-07032.

T-27 Mechanisms in Teratogenesis Studied With Whole Rat Embryos in Culture, T.J. FLYNN, Division of Toxicological Studies, Food and Drug Administration, 8501 Muirkirk Road, Laurel, MD 20708-9801.

The post-implantation rodent embryo culture system developed by Denis New and colleagues during the 1970s has been used extensively in studies in experimental teratology. While much work has been directed toward development of rodent embryo culture as a screening assay for environmental teratogens, the major value of this system lay in its ability to study the effects of agents directly on embryos without potential confounding maternal factors. Types of mechanistic studies that have been conducted with cultured embryos include: elucidation of active species or metabolites; effects of enzyme inhibition; gene-regulation by developmental toxicants; the role of anti-embryo antibodies in developmental toxicity; structure-activity relationships; and, effects of maternal nutritional status. Recent studies in our laboratory have focused on mechanisms by which normal body laterality is determined in developing rat embryos. Our cultured embryos are, presently, experiencing a high (50%) incidence of spontaneous laterality inversions (situs inversus) of unknown etiology. We have ruled out as causes genetic factors and adrenergic stimulation, presently the only positively identified causes of situs inversus. We are also studying rat embryos cultured in human serum specimens as biomarkers for possible nutritional factors resulting in infertility.

T-28 T. Rick Irvin
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Postimplantation Embryo Culture Systems for Mechanistic Studies of Prenatal Toxins

NO ABSTRACT SUBMITTED

T-29 In vitro Embryotoxicity Test Using Blastocyst Derived Euploid Embryonal Stem (ES) Cells of the Mouse.
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During the last decade several groups have tried to use cellular systems for the prediction of developmental toxicity. According to the results mammalian cell culture systems seem to be more promising than non-mammalian systems. Moreover, primary cell cultures of embryonal cells seem to be more suitable than permanent cell lines including permanent human cell lines from various embryonic tissues.
To overcome these shortcomings we used a pluripotent embryonic stem cell line (ES cells) derived from mouse blastocysts. ES cells can be maintained in the undifferentiated state by cultivation on a feeder of embryonic fibroblasts. In suspension culture ES cells spontaneously differentiate into complex organized embryoid bodies resembling early preimplantation embryos. Culture conditions could be established to allow a reproducible differentiation of ES cells. Formation of spontaneously beating cardiac muscle, nerve and blood cells as well as cartilage can be analysed by morphological and biochemical parameters. Preliminary results indicate that this complex mammalian culture system based on ES cell differentiation holds promise for in vitro embryotoxicity testing.

T-30 In vivo and in vitro Comparisons of Mutational Changes. B.W. GLICKMAN. Centre for Environmental Health, University of Victoria, BC Canada

Mutation research has taken a new twist with the development of cloning and sequencing technology. It is now possible to execute experiments which analyze the nature and location of mutation at the DNA level. A collection of such sequenced mutations has been termed a "mutational spectrum". As mutational spectra reflect the deposition of DNA damage and the action of error-avoidance mechanisms, mutational spectra have been found to be unique for each class of mutagen. Moreover, it is now possible to compare mutational spectra obtained using the same genetic target in bacteria in vitro, in bacteria in host-mediated assays (i.e., as bacteria in mice) and in transgenic animals! Similarly, it is possible to study a mammalian gene in its endogenous location, in cells transformed with the same gene on a shuttle vector as well as directly in people in vivo. Here we report that diverse systems often yield the same or very similar results in terms of the mutational response to the environment. An example of such a situation is ultra-violet light. In some cases however, differences are noted. One example is benzo[a]pyrene diolepoxide (BPDE) which produces somewhat different spectra in bacteria than in mammalian cells. In this case differences in DNA repair capacity are likely to be responsible. Examples of cases of both similarities and differences will be explored, but the take home lesson is that in vitro studies do provide insights into mutational mechanisms.

T-31 Malignant Transformation of Human Fibroblasts in Vitro. J. J. MCCORMICK and V. M. Maher, Carcinogenesis Laboratory, Michigan State University, East Lansing, MI 48824-1316.
Normal human cells have been refractory to malignant transformation in vitro. To study this problem, we developed an infinite life span, diploid, human fibroblast cell strain (MSU-1.0). These cells express a transfected v-*myc* oncogene and have one or more additional changes. From MSU-1.0 cells, we developed the MSU-1.1 strain, which has a stable, near-diploid karyotype, composed of 45 chromosomes including two marker chromosomes. MSU-1.1 cells can be transformed to malignancy by transfection of an H-, K-, or N-*ras* oncogene. The malignant cells exhibit the stable karyotype of the parental MSU-1.1 cells. We also have shown that malignant variants of MSU-1.1 cells can be induced by carcinogen treatment. Exposure to carcinogen caused a dose-dependent increase in foci formation, and cells from such foci grew to a higher density in medium containing 1% serum than did the MSU-1.1 cells they were derived from. A substantial fraction of the focus-derived strains formed large-sized colonies in agarose at high frequency and proved to be malignant. The focus-derived transformants each exhibited unique chromosome changes in addition to the marker chromosomes of the parental strain. These data suggest that carcinogen-induced oncogene activation (or tumor suppressor gene inactivation) commonly takes place as a result of major chromosome alterations. Although most of the tumors were spindle cell sarcomas, we have also observed myxoid, giant cell, and round cell sarcomas, as well as benign tumors. Taken all together, these data indicate that at least six independent changes are required for human fibroblasts to become malignantly transformed. (Supported by DHHS Grant CA21289, NIEHS Contract ES65152, and DOE Grant ER60524)

T-32 CYTOGENETIC APPROACH TO DOCUMENT FACTORS THAT CONTRIBUTE TO THE DEVELOPMENT OF CANCER. W.W. Au, Department of Preventive Medicine and Community Health, The University of Texas Medical Branch, Galveston, TX 77550.

It is well documented that cancer is a complex process involving multiple and sequential changes in the clonal evolution of abnormal cells. The cause of these changes are under intensive investigation. Studies using cells in culture may be useful in elucidating the mentioned phenomenon. In one of our studies, we have used an in vivo/in vitro mouse mammary tumor model for our investigation. Mammary glands from irradiated mice which would later develop mammary tumors were used to set up cell cultures. In our sequential analyses, we observed that extensive chromosome changes in number and in structure occurred during early passages of these cells. Subsequently, cells having stabilized but abnormal karyotypes evolved. These were followed by alteration of the retinoblastoma tumor suppressor gene and then by amplification of the c-myc oncogene before the cells became tumorigenic upon injection into mice. In another study, we developed a challenge assay to investigate infidelity of DNA repair. We observed that non-clastogenic and non-cytotoxic doses of carcinogens can cause infidelity of DNA repair. We hypothesize that low doses of carcinogens can cause repair problems that lead to the existence of an unstable genome. This unstable genome allows the accumulation of multiple and sequential genetic changes which can lead to the development of cancer.

T-33 Cultured Human Oral Epithelium: Growth, Transformation and Tobacco-Related Pathology.
R. GRAFSTRÖM, K. Sundqvist, P. Kulkarni and Y. Liu. Department of Toxicology, Karolinska Institutet, S-104 01 Stockholm, Sweden.

Human oral epithelial cell cultures can be grown and transferred in serum-free MCDB 153 medium. The cells express keratins and typical structural features, exhibit up to 40% colony-forming efficiency, divide at about 1 population doubling (PD) per day on fibronectin-collagen-coated dishes and can undergo more than 65 PD. From measurements of growth rate, colony forming efficiency, cell surface area, migration and synthesis of involucrin and cross-linked envelopes, growth is increased by epidermal growth factor, cholera toxin, retinoic acid and pituitary extract and inhibited by transforming growth factor β_1 (TGF-β), Ca^{2+}, 12-O-tetradecanoylphorbol-13-acetate and serum; the latter 3 agents also accelerate terminal differentiation. Gene transfer and expression of SV40 large T antigen (SV40T) extends the life span of the cells to about 200 days and seem to have immortalized one cell line which has been in culture for more than a year. Karyotype abnormalities occur in SV40T-transfected cells and a buccal squamous carcinoma cell line (SqCC/Y1). To a variable extent, the SV40T-transfected and carcinoma cells show resistance to the growth-modulating effects of TGF-β and serum. The carcinoma cell line forms progressively growing tumors in athymic nude mice, whereas none of the SV40T-transfected lines produced tumors. The tobacco-specific carcinogen 4-(metyl-nitrosamino)-l-(pyridyl)-1-butanone is metabolized in explant and epithelial cell cultures. Moreover, normal cells are more sensitive to the toxicity of tobacco extracts than transformed cells. Carcinogenesis studies are now possible in explant cultures and normal, experimentally transformed and tumorous human oral epithelial cells grown at defined culture conditions.

T-34 Designing an In Vitro Screening Battery to Evaluate Pesticide Neurotoxicity, B. VERONESI, U.S. Environmental Protection Agency, HERL, Neurotoxicology Division, Cellular and Molecular Toxicology Branch, MD74B, Research Triangle Park, NC, 27711

The need to develop, validate and utilize *in vitro* models for neurotoxicity testing is widely appreciated. This presentation will detail the strategy and techniques used in defining a screening battery to address the relationship between cholinesterase inhibition (CHI) and neurotoxic outcome following pesticide exposure. This screening battery has been design to address critical questions that are either elusive or cost-ineffective in whole animal models. Issues such as defining "adverse effect" for pesticide neurotoxicity; identifying pesticides from among other neurotoxic and non-neurotoxic chemicals using neural-specific endpoints; establishing dose-response data using both CHI and cytotoxicity endpoints; differentiating neuropathy-causing (OPIDN) pesticides from CHI using differential neurotoxic endpoints; establishing structure-activity-relationships within pesticide groups; and characterizing the effects of pesticides on "neuromuscular junction" co-cultures will be discussed. Topics will include technical considerations in the design of the battery, choice of appropriate cell models and endpoints, automated data collection systems, and the validation process. Preliminary data and its relevance to in vivo models of pesticide toxicity will be presented.

T-35 The CytoFluor 2300 Fluorescent Plate Reader and a Multiple Endpoint Assay Reveals Neuroprotective Abilities of Nimodipine.
A. Danks, R. Isaacson, J. Im, and R.G. VAN BUSKIRK. State University of New York, Binghamton, NY 13902-6000.

Loss of calcium homeostasis is thought to be a possible mediator of both programmed cell death and cellular necrosis. A variety of calcium channel antagonists are known to prevent neuronal death in vitro and it is presumed that the neuroprotective properties of these agents are due to their abilities to decrease intracellular calcium. We used a multiple endpoint assay to determine the mechanisms by which one of these voltage-sensitive calcium channel blockers, nimodipine, might protect neurons against toxic insult. An equal molar concentration of ouabain and monensin (O/M) was used as a toxic regime and resulted in an increase in intracellular calcium in a differentiated septal cell line as revealed by the calcium indicator dye, Fluo3-AM. Lysosomal function was also compromised with the addition of O/M as reflected by decreased neutral red incorporation. Similarly, O/M increased free radical formation in a dose-dependent manner as indicated by 2,7 - dichlorofluorescin. A nimodipine pre-incubation ameliorated the cytotoxic effects on lysosomes and decreased free radical formation, but nimodipine augmented the O/M increase in intracellular calcium. Thus the CytoFluor 2300 and the multiple fluorescent assay revealed that nimodipine is neuroprotective but at least some of the neuroprotective abilities of this calcium channel antagonist might be independent of this drug's ability to alter intracellular calcium.

T-36 Effects of Lead on Ion Channels in Mouse N1E-115 Neuroblastoma Cells. J. VAN DEN BERCKEN, H.P.M. Vijverberg, M. Oortgiesen and T. Leinders, Research Institute of Toxicology, Utrecht University, P.O. Box 80.176, NL 3508 TD Utrecht, The Netherlands

Mouse neuroblastoma cells of the clone N1E-115 contain a variety of well-characterized voltage-dependent and receptor-activated ion channels. N1E-115 cells are therefore very suitable to study the differential effects of neurotoxicants on various types of ion channels in the same cell type under the same experimental conditions.
Recently we have studied the effects of inorganic lead (Pb^{2+}) on N1E-115 cells by means of the whole cell and single channel patch clamp technique.
Low concentrations of Pb^{2+} (1 nM - 3 μM) markedly reduce the nicotinic inward current induced by acetylcholine (ACh). At higher Pb^{2+} concentrations (10 - 100 μM) the blocking effect is, however, reversed. This dual effect of Pb^{2+} can be described by the sum of two sigmoidal concentration-effect curves, with an IC50 value of 19 nM and an EC50 of 21 μM, respectively. The inward current induced by serotonin (5-hydroxy-tryptamine, 5-HT), which is mediated by an independent population of 5-HT3 receptors, is much less sensitive to Pb^{2+} (IC50 = 50 μM). Calcium channels are blocked by Pb^{2+} at micromolar concentrations (IC50 = 5 μM), while sodium channels are not affected (IC50 > 100 μM). Pb^{2+} at micromolar concentrations also differentially activates two types of calcium-activated potassium channels, SK and BK channels (EC50 < 1 μM and > 1 μM, respectively).
These results support the hypothesis that Pb^{2+} affects neurotransmitter release by blocking voltage-dependent calcium channels in the presynaptic nerve ending. They further demonstrate that the neuronal type nicotinic ACh receptor-ion channel complex is most sensitive to Pb^{2+}, as it is selectively blocked by nanomolar concentrations of Pb^{2+}.
Supported by the Netherlands Organization for Scientific Research NWO and by Shell Corporation.

T-37 In Vitro Evaluation of Neural Function. T.K. ROWLES, D. Taylor, A. Nostrandt, and M. Ehrich. Department of Animal Science, University of Tennessee, Knoxville, 37901; Department of Biomedical Sciences, Virginia Tech, Blacksburg, Virginia, 24061.

We are evaluating morphology and neural cell function after toxicant exposure using cultures and co-cultures of neuronal, glial and microvascular endothelial cells. Normal neural function is dependent on the neuronal microenvironment, as influenced by the microvascular endothelial cells and glial cells, and the neuronal cell viability.

Neuronal cytotoxicity was evaluated in human SH-SY5Y neuroblastoma cells using microtiter assays for viability (tetrazolium dye, rhodamine 123, and dye uptake/exclusion studies), microtiter assays of esterase function, and morphological examinations using light microscopy and time-lapse videomicroscopy. The non-neuronal components of the central nervous system, such as astoroglia and brain microvascular endothelial cells, were also evaluated for viability.

Differentiated SH-SY5Y cells exposed to neurotoxicants, such as the organophosphorus ester mipafox, demonstrated differences in cytoplasmic membrane activity and cytoplasmic morphology. Inhibition of esterase activities were also detected early after organophosphorus ester exposure. Both the tetrazolium dye and rhodamine 123 test were sensitive early indicators of toxicity following exposure of SH-SY5Y cells to lead and cadmium.

The tetrazolium dye test (MTT) has been useful in detecting cytotoxicity in cell lines of endothelial cells and astroglia, however the MTT test has not worked as well with primary cultures of these non-neuronal cell types. We have, however, been able to evaluate the permeability of the endothelial cell layer in co-culture configuratoin with astroglia using a fluro-dextran.

This work was supported by the Center for Alternatives to Animal Testing and USDA Hatch Funds.

V-1 Expression of P-glycoprotein in Normal and Malignant Cells.
G. Bradley, S. Rajalakshmi and V. Ling. Ontario Cancer Institute and Department of Pathology, University of Toronto, 500 Sherbourne Street, Toronto, Canada, M4X 1K9

P-glycoprotein (Pgp) is overexpressed in many mammalian cell lines that have been selected for multidrug resistance. Transfection of drug-sensitive cells with cDNA's encoding Pgp results in acquisition of a multidrug-resistant phenotype by the recipient cells. In vivo, Pgp is detected at high levels in several specialized mammalian cell types and also in clinical tumor samples. Over-expression of Pgp has been found in tumor samples both at initial diagnosis and upon relapse from chemotherapy. We have investigated the role of Pgp in carcinogenesis by studying the Orotic Acid model of rat hepatocarcinogenesis. By immunohistochemical staining of preneoplastic and neoplastic liver lesions, we have found that Pgp expression correlated with a trabecular-sinusoidal type of tissue architecture which increased in prominence during hepatic tumor progression. Furthermore, in rats which developed extensive lung metastases, Pgp was consistently detected in every metastatic lesion. These results suggested that, during rat hepatocarcinogenesis, increased expression of Pgp is associated with a more malignant phenotype. The molecular basis of overexpression of Pgp during hepatic tumor progression has not been determined. Cellular events during carcinogenesis as well as factors from the microenvironment may modulate Pgp expression in the malignant hepatocyte. The relative importance of potential modulating factors may be assessed by studying malignant hepatocytes in culture systems. These studies may ultimately aid in our understanding of the basis of de novo PgP overexpression in patient's tumors.

V-2 Molecular Analysis of the Multidrug Transporter.
M. M. GOTTESMAN, Laboratory of Cell Biology, National Cancer Institute, NIH, Bethesda, MD 20892.

The simultaneous resistance of human cancers to multiple natural product anti-cancer drugs results frequently from expression of a 170,000 dalton molecular weight multidrug efflux pump (P-glycoprotein) encoded in the human by the *MDR*1 gene. P-glycoprotein consists of 1280 amino acids with 12 transmembrane domains and 2 ATP binding/utilization sites. The homology of these ATP sites to similar sites in many other transporters defines a superfamily of ATP-dependent proteins including the cystic fibrosis transmembrane regulator (CFTR). Normal tissues appear to use P-glycoprotein for transepithelial excretion of toxic materials (liver, kidney, intestine), as a barrier to uptake of toxic materials (capillaries in brain, testes, placenta), and to handle steroids (adrenal cortex). Human cancers appear to express P-glycoprotein for three reasons: (1) Because they are derived from tumors which normally express P-glycoprotein (e.g., colon, liver, kidney, adrenal and pancreatic cancers; (2) Because they have been selected for multidrug resistance during chemotherapy (e.g., leukemias, lymphomas, breast and ovarian cancers); and (3) Because some of the genes which are associated with malignant progression (i.e., mutant p53, *ras*) turn on expression of the *MDR*1 gene (e.g., CML in blast crisis, some leukemias and various metastatic cancers). The broad resistance encoded by the *MDR*1 gene has made it possible to protect bone marrow from toxic effects of chemotherapy in transgenic mice and after infection with *MDR*1 retroviruses.

V-3 **Altered DNA Topoisomerase II in Multidrug Resistance.**
W. T. BECK, M. K. Danks, J. S. Wolverton, M. Chen, B.Y. Bugg, D. P. Suttle, St. Jude Children's Research Hospital, Memphis, TN 38101.

The characteristic feature of multidrug resistance (MDR) associated with drugs that interact with DNA topoisomerase II (topo II) is *a*lterations in *t*opo II activity or amount (at-MDR). We have characterized the at-MDR phenotype in human leukemic CEM cells selected for resistance to the topo II inhibitor, VM-26. Compared to drug-sensitive cells, the key findings are that at-MDR cells exhibit (i) decreased topo II activity; (ii) decreased drug sensitivity, activity and amount of nuclear matrix topo II; (iii) increased ATP requirement of topo II; (iv) decreased topo II phosphorylation; and (v) a single base mutation in topo II resulting in a change of Arg to Gln at position 449. Recent results using single-stranded conformational polymorphism analysis confirms the presence of this mutation in these and at least one other cell line expressing the at-MDR phenotype. Finally, we have observed marked changes in the nuclear distribution of topo II in cells treated with anti-topo II drugs and have also found these changes to be attenuated in drug-resistant cells. We postulate that stabilization of DNA-topo II complexes by drug-treatment results in covalent attachment of the enzyme to the DNA, protecting it from proteolysis and permitting detection of more topo II molecules. We propose that MDR associated with alterations in topo II may have clinical consequences, and our current efforts involve exploiting these observations in the development of probes that may be useful to identify such drug resistant cells in the tumors of patients. Results of these and other studies will be reported. (Supported in part by research grants CA-30103, CA-40570, and CA-47941, CORE grant CA-21765, all from NCI, Bethesda, MD, and in part by ALSAC)

V-4 IN VITRO AND IN VIVO RESISTANCE IN L1210 MURINE LEUKEMIA CELLS TO NOVEL ANTITUMOR COMPOUNDS.
M. Grandi, E. Pesenti and C. Geroni. Farmilalla Carlo Erba, Erbamont Group, R&D/Oncology Lab., Nerviano (Milano), Italy.

We have isolated and cloned two murine leukemia L1210 cell lines resistant to the novel antitumor agents FCE 23762 (methoxymorpholinyl doxorubicin, MMRDX) and FCE 24517 (benzoylmustard derivative of distamycin A). Both cell lines do not present the multidrug resistant (mdr) phenotype and possess a mechanism of resistance specific for the selecting agent and derivatives of the same chemical class. L1210/MMRDX cells are resistant in vitro and in vivo to anthracycline derivatives bearing the methoxymorpholinyl group; resistance to the corresponding morpholinylanthracyclines is observed only in vivo, and in vitro after microsomal activation. L1210/24517 cells are resistant in vitro and in vivo to distamycin A derivatives bearing different reactive moieties and to distamycin A itself, although at higher drug concentrations. The two resistant sublines represent a useful tool to elucidate the modes of action of the two drugs; moreover, they can be of help in addressing the synthesis of novel not-cross resistant derivatives.

V-5 **Manipulating Cellular Drug Resistance with Genetic or Serological Reagents.** I.B. Roninson, A.V. Gudkov, C. Zelnick, A.R. Kazarov, T.A. Holzmayer, and E.B. Mechetner. Department of Genetics, University of Illinois at Chicago, Chicago, IL 60612, U.S.A.

Selective inhibition of specific genes can be accomplished through the introduction of genetic suppressor elements (GSEs) encoding antisense RNA or dominant negative mutant proteins. We have developed a general strategy for producing biologically active GSEs by expression selection of random fragments of the DNA targeted for suppression. This procedure was initially tested on bacteriophage lambda, where it revealed some previously unknown properties of this well-studied virus. The same approach was used in mammalian cells to isolate GSEs conferring resistance to drugs whose cytotoxicity is mediated by topoisomerase II (topo II) (in collaboration with D.P. Suttle and W.T. Beck). Etoposide selection was used to obtain GSEs from a retroviral library carrying random fragments of human topo II (α form) cDNA. The isolated GSEs encoded either short antisense RNAs or peptides corresponding to several different regions of topo II cDNA. Expression of the antisense- and of some of the sense-oriented GSEs caused a decrease in the intracellular levels of topo IIα. In addition to etoposide, cells expressing topo II-derived GSEs were resistant to teniposide, amsacrine and doxorubicin, but not to actinomycin D or to several drugs whose activity has not been linked to topo II. Other applications of the GSE approach will be discusssed. We will also describe the inhibition of P-glycoprotein (Pgp)-mediated multidrug resistance (MDR) by a monoclonal antibody, UIC2, which reacts with an extracytoplasmic epitope of human Pgp. UIC2 inhibits the efflux of Pgp substrates from MDR cells and significantly increases the cytotoxicity of Pgp-transported drugs. Potentiation of cytotoxicity by UIC2 was observed with all the tested drugs associated with MDR, but not with any of the drugs to which MDR cells are not cross-resistant. UIC2 or its derivatives may provide an alternative to chemical agents for the reversal of MDR in clinical cancer.

V-6 Reversal of Adriamycin (Adr) Resistance by Lontidamine (LND) in a Human Breast Cancer Cell Line.
G. CITRO[1], I. D'Agna[1]* and G. Zupi[1], [1]Ist. Regina Elena; *Ist. Tecnologie Biomediche, CNR; Rome, ITALY.

LND, a compound able to affect membrane permeability as well as energy metabolism, was studied as possible agent in reversal of Adr resistance. Two human breast cancer cell lines, MCF7 wild type and its derivative Adr-Resistant (MCF7 Adr-R), were exposed to LND administered alone or in combination with Adr for different times and doses. The results demonstrate that LND enhances the lethal effect of Adr on both lines. However, the enhancement of Adr cytotoxicity induced by LND is more significant in Adr-R line than in MCF7 wild type. The reversal of Adr resistance, evident from the significant decrease of the resistance index, is accompanied by the increase in intracellular Adr content. MCF7 Adr-R cells treated with the LND-Adr combination show a doubled intracellular Adr amount compared to the cells exposed to Adr alone. In order to better define the role of plasma membrane in the LND-mediated reversal of MDR, the membrane potential of the two cell lines was measured by flow cytometry before and after LND treatment. The results demonstrate that: a) the plasma membrane of the resistant cells is depolarized compared to that of the sensitive cells; b) depending on the exposure time, LND alters the membrane potential in the resistant line only. The LND effect can possibly be related to membrane modifications of the resistant phenotype or to changes in the activity of the GP170, which is overexpressed in the MCF7 Adr-R cells.

V-7 Studies on MDR Variants of Human Lung Carcinoma Lines.
M. CLYNES, A. Redmond, E. Moran, National Cell & Tissue Culture Centre/BioResearch Ireland, Dublin City University, Glasnevin, Dublin 9. Ireland.

Multidrug resistant variants of 3 human non-small cell lung carcinoma lines have been selected by adaptation to progressively increasing concentrations of adriamycin. The variants derived from 2 squamous cell carcinoma lines designated DLKP-A and SK-MES-1-A show 400-fold and 120-fold resistance to adriamycin, respectively and adenocarcinoma variant SK-LU-1-A shows 100-fold resistance.

Drug resistance is a stable property of the cells, which can be grown for at least 3 months in the absence of drug without loss of resistance. DLKP-A and SK-MES-1-A show significant levels of cross-resistance to VP-16, VM-26, Vincristine and Colchicine but SK-LU-1-A is cross-resistant only to the latter two drugs. P-Glycoprotein over-expression appears to be one determinant of resistance in DLKP-A and SK-MES-1-A based on Western blotting, immunofluorescence, antisense transfection studies and verapamil reversal of resistance. Since the mechanisms of drug non-responsiveness of non-small cell lung carcinoma *in vivo* remain poorly understood, further studies on these variants may provide information relevant to treatment of this disease.

V-8 Olfactory Organ Culture In Vivo and In Vitro.
P.P.C. GRAZIADEI, Department of Biolgoical Science, Florida State University, Tallahassee, FL 32306 U.S.A.

The olfactory organ has several unique characteristics offering an opportunity for the understanding of problems of general neurobiology. For instance: 1) its sensory neurons undergo turnover even in adult animals, 2) the sensory axons form the typical glomerular terminal structures in the absence of the target, 3) the presence of these axons induces profound changes (hyperplasy/hypertrophy) when they reach several CNS regions, 4) the absence of the organ induces profound hypoplasy of the forebrain in amphibia; several, albeit less profound changes occur in the brain of other vertebrates, 5) the organ produces, besides the sensory neurons, other CNS neurons (the LHRH neurons), thus playing a key role in the sexual development of the animal. The culture of the olfactory organ in vitro or in oculo has been implemented in several laboratories and three main lines of research can be outlined: a) after removal of the olfactory organ (at late embryonic to early postnatal stages) its cellular components are isolated and selectively dissociated; neuron precursors are then plated for cell physiology studies, b) the olfactory organ is cultivated in toto (organ culture) for the study of the differentiation of its pluripotent neurogenetic matrix, c) the olfactory organ is cultivated in vitro or in oculo alone or in conjunction with other CNS regions to study phenomena of trophic interaction and the putative factors involved.

The above mentioned approaches and their most recent results will be described and critically discussed; new avenues of research will be outlined for discussion. (supported by NIH NS 20699)

V-9 Peripheral Aspects of Odor Perception
P. PELOSI, Istituto di Industrie Agrarie, via 5, Michele, 4 - 56124 Pisa, Italy.

The perception of odors seems to be mediated by soluble proteins, abundant in the nasal mucus of vertebrates. They include odorant-binding proteins (OBPs), odorant metabolizing enzymes and recently discovered glycoproteins, such as olfactomedin and vomeromodulin. The bovine OBP was the first to be purified and characterized both in terms of chemical structure and ligand-binding specificity. Other members have been isolated from different species of vertebrates. Despite the great amount of data, accumulated in the last ten years, their physiological function is still unclear. The poor binding specificity and the fact that a single type of OBPs had been identified seemed to exclude a discriminating role in olfaction. Being members of a superfamily of carrier proteins, OBPs have been proposed to take odorants to the membrane receptors or away from them. The recent discovery of a second class of OBPs in the nasal mucosa of some vertebrates has reopened the question of whether some odor discrimination could also occur at the level of soluble proteins of the mucus. In insects, where a similar system, using soluble proteins in the mechanism of odor perception, two classes of pheromone-binding proteins (PBPs) and two of general odorants-binding proteins (GOBPs) have been described. It is also interesting that proteins homologous to OBPs are known to be present in media used by many vertebrates for chemical communication, such as the urine and the vaginal secretion. The research in these parallel fields can provide useful information towards the understanding of the physiological role of OBPs.

V-10 Molecular Mechanisms of Olfactory Signal Transduction.
H. BREER, I. Boekhoff, J. Krieger, K. Raming, J. Strotmann, and E. Tareilus Institute of Zoophysiology, University Stuttgart-Hohenheim, 7000 Stuttgart 70, FRG

Several lines of evidence suggest that the chemo-electrical signal transduction process in olfactory receptor cells involves second messenger cascades. To demonstrate that upon odorant stimulation the levels of second messengers actually changes in a time range relevant for primary processes, a rapid kinetic methodology was applied monitoring the odorant-induced formation of second messengers in the subsecond time range. A very rapid increase in the second messenger (cAMP or IP_3) concentration was detected after about 50 msec; thereafter the signal rapidly attenuated to the basal level within 100-200 msec. Thus, the induced molecular signal clearly preceeds the generator currents of the receptor cells. The transient nature of the signal is apparently due to rapid desensitization phenomena mediated by specific protein kinases. Experiments using specific inhibitors have indicated, that the cAMP pathway in terminated by protein kinase A, whereas the IP_3 cascade is attenuated via protein kinase C activity. It is presently unclear if receptor proteins are phosphorylated. The observation that odorant-induced second messenger formation requires active G-proteins suggests that the reaction cascade is initiated by an interaction of odorants with receptor proteins belonging to the superfamily of G-protein-coupled receptors. Based on recent work by Buck and Axel and using PCR-amplification procedures a number of putative odorant receptors from rat and salamander have been cloned. For some of the receptor subtypes the sites of expression in the olfactory epithelium were revealed using in situ hybridisation techniques. The pattern of expression corresponds to groups of olfactory neurons topographically distributed within the nasal neuroepithelium.
Supported by the Deutsche Forschungsgemeinschaft

V-11 REGULATION OF OLFACTORY NEURON GENE EXPRESSION
F.L. MARGOLIS, ROCHE INST. MOLEC. BIOL., NUTLEY, N.J. 07110
Olfactory receptor neurons (ORN) are located in the nasal vault and are thus vulnerable to environmental agents. In response, the olfactory neuroepithelium preserves precursor cells capable of replacing these ORN throughout life. Thus, although this tissue contains only a single neuronal cell type, various developmental stages of the ORN coexist in the same animal. For example, mature ORN express olfactory marker protein (OMP), an abundant, 19kD cytoplasmic protein. In contrast, immature ORN express B50/GAP43 a growth related, calmodulin binding membrane phosphoprotein. The ORN precursors express neither OMP nor B50/GAP43. These observations illustrate an ordered sequence of gene expression resulting in changing cellular phenotype during ORN development. Changes in temporal and spatial patterns of cell specific gene expression determine cellular phenotype and derive from the interplay between genetic and epigenetic processes. To study these events in ORN we are using both in vivo and in vitro assays to characterize the regulation of OMP expression. To identify and study the specific genomic elements involved in the regulation of OMP expression we are using *in vivo* analyses in transgenic mice and *in vitro* gel mobility shift assays and DNase footprinting to identify loci of functional DNA-protein interaction. We have identified two homologous DNA motifs upstream of the OMP gene that react with a factor we have named OLF-1. This factor appears to be developmentally regulated and tissue specific. Analyses of transgenic mice indicate that one OLF-1 site is insufficient to drive reporter gene expression but a construct containing both sites directs reporter gene expression to OMP-expressing ORN. OLF-1 may represent an olfactory specific trans-acting factor capable of interacting not only with the gene for OMP but with other ORN specific genes as well. As such it may participate in regulating the expression in ORN of genes associated with olfactory signal transduction.

V-12 A Molecular Cytogenetic Assessment of Structural and Numerical Aberrations Associated with Human Cancers
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Human malignancies are now thought to progress toward metastasis through accumulation of genetic aberrations. Fluorescence in situ hybridization (FISH) with probes to the aberrant regions is proving to be a powerful tool for detection and characterization of the aberrations since it allows a) analysis of individual interphase cells so that cell culture is not needed, b) detection of aberrant cells even when present at low frequency, c) analysis of the genetic nature of the aberrations and d) simultaneous analysis at several loci. We illustrate the potential of this approach in cancer diagnosis and biology by showing its application to analysis of human ovarian and breast cancers. Specifically, we: a) Describe the quantitative analysis of chromosome copy number and suggest the potential utility of copy number heterogeneity as a prognostic indicator, b) Show that ERBB2 and CMYC amplification and heterogeneity therein can be assessed accurately and suggest the possibility of prognostication based on the most heavily amplified subpopulation, c) Illustrate that loss of heterozygosity (LOH) on chromosomes 3p, 16q and 17p frequently is caused by physical deletions that can be detected using FISH and we suggest the utility of this approach in prognostication and in definition of the regions of common LOH and d) Show the use of FISH for study of the mechanisms involved in aberration formation.

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NO ABSTRACT SUBMITTED

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NO ABSTRACT SUBMITTED

V-15

Identification of Human Haemopoietic Stem Cells (HSC)

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Various classes of haemopoietic progenitors have been identified and characterized by their phenotype, as well as by their properties in vitro and in vivo. It is thus possible to distinguish a population of committed progenitors, capable of forming erythroid (BFU-E), granulomacrophagic (CFU-GM) and mixed (CFU-Mix) colonies, which is CD38+,CD34+, while a more primitive, pluripotent population is characterized by a CD38- CD34+ phenotype (Weiss et al. 1991).

Another property, i.e. the capacity to sustain haemopoiesis in long-term cultures, has been found to be prevalent in a population of CD34+,Rh-,HLA-DR± cells, present both in bone marrow and in peripheral blood (Udomsakdi et al. 1991). It is of interest that human umbilical cord blood cells are superior to marrow-derived cells in the long-term culture system.

The relationship among these HSC classes is however not clear and it has been suggested that they represent a sequential activation of stem cell clones (Abkovitz et al. 1990). The complex nature of HSC's is therefore a matter of further investigation and its understanding is essential for future developments in basic reasearch and in the field of clinical transplantation.

V-16 Expression of Genes in Murine Bone Marrow Fractions Enriched for Pluripotent Hemopoietic Stem Cells.

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The regulation of the differentiation and replication of hemopoietic stem cells was studied by application of the PCR-based total cDNA cloning technique (NAR 17: 2919) on purified stem cell fractions (PNAS 88: 7420). In vitro amplified cDNA libraries were constructed from sorted quiescent stem cell-enriched fractions of murine bone marrow (the Rhodamine 123-dull and -bright fractions of WGA-positive, GM-depleted suspensions), and from Rh123-bright cells isolated after culturing the Rh123 dull cells for 1 day in the presence of IL-3 and CSF-1, as well as from LPS-activated spleen B lymphocytes. Subtraction cloning revealed so far two genes expressed in Rh123 dull, but not in Rh123 bright cells and lymphocytes, and several other genes expressed in both Rh 123 dull and bright cells, but not in lymphocytes. Amplification of cDNA with degenerate oligonucleotides has revealed expression of at least 10 different protein kinases (several of which were not reported previously) in the stem cell-enriched fraction. Four of these are receptors for as yet unknown growth factors or other biological reponse modifiers. In addition, semi-quantitative PCR techniques with primers specific for the newly detected genes are applied to cDNA from sorted, phenotypically pure cells from the lymphoid, erythroid and myeloid differentiation lineages and the observed expression patterns are compared with those in the highly enriched and cultured stem cell fractions. As expected, both up and down regulation as well as transient expression patterns are found during hemopoietic differentiation.

V-17 The Generation of Human Colony-Forming Cells (CFC) and the Expansion of Hematopoiesis In Vitro is Dependent on the Presence of Stem Cell Factor (SCF)
A.R. MIGLIACCIO, G. Migliaccio, G.C. Mancini, K. Zsebo and J.W. Adamson. New York Blood Center, 310 E. 67 St., New York, NY 10021 and Amgen, 1900 Oak Terrace Lane, Thousand Oaks, CA 91320.

We have analyzed the effect of SCF, in combination with other growth factors, on the generation of CFC and on the expansion of hematopoiesis in vitro from light density, soybean agglutinin$^-$, CD34$^+$ cord blood cells under serum-deprived conditions. No growth factor, alone, generated CFC or expanded hematopoiesis under these conditions. However, SCF, in combination with IL-3 or with "late-acting factors" (G-CSF or Epo), generated large numbers of mature cells as well as CFC for up to one month (from <160 CFC/culture at day 0 to >3000 CFC at day 10). The lineages of the mature cells and the types of CFC generated varied with the different growth factors. In the presence of SCF plus IL-3, BFU-E and GM-CFC were generated and all the lineages were present among the differentiated cells. In contrast, in the presence of SCF and G-CSF or Epo, the progenitor cells as well as the differentiated cells generated were dictated by the late-acting growth factor (i.e. mostly G-CFC and myeloid cells in the presence of SCF and G-CSF vs. BFU-E, CFU-E and erythroblasts in the presence of SCF and Epo). "Early acting" factors, such as IL-1 and IL-6, were relatively ineffective, even in combination with SCF and were unable to increase CFC numbers under these conditions. Thus, marked expansion of erythropoiesis and granulopoiesis can be achieved in vitro by as few as two factors - SCF acting as the early factor along with the appropriate late-acting factor.

V-18

HEMATOPOIETIC DIFFERENTIATION OF EMBRYONIC STEM (ES) CELLS IN CULTURE
Marion Kennedy, Michael Wiles* and Gordon Keller; The National Jewish Center, Denver, CO, USA, and *The Institute for Immunology, Basel, Switzerland

When cultured in methyl cellulose in the absence of feeder cells and LIF, ES cells generate embroid bodies (EBs) which consist of differentiated cells from various lineages including those of the hematopoietic system. Approximately 50% of the cells from the CCE ES line will give rise to an EB in semi solid media, and of these, greater than 90% contain hematopoietic cells, as demonstrated by the presence of clonable precursors. Hematopoietic precursors can be detected within 5 days of differentiation in the absence of added growth factors, with the exception of those present in fetal calf serum (FCS) or those produced within the EBs. The first detectable precursors (day 5) are erythropoietin$^+$(Epo) responsive and generate small colonies of erythoid cells. Many of the cells within these colonies are nucleated, and at least a subpopulation produce embryonic globin RNA, suggesting that they represent the primitive erythroid lineage. Within the next 24 hours, a second population of eythroid precursors as well as macrophage precursors can be detected within the EBs. These erythroid precursors require both kit ligand (KL) and Epo for growth and give rise to cells that express predominantly adult globin RNA. Precursors of the neutrophil and mast cell lineages develop within the following 3-4 days of differentiation.

Preliminary studies indicate that precursors able to generate colonies containing cells with a lymphoid-like morphology develop late within the differentiation cultures. A continuously growing cell line which expresses both B220 and Thy-1.2 has been established from one of these colonies. These cells do not express cytoplasmic or surface immunoglobulin (Ig), T-cell receptors (TCR), CD4, CD8, or Mac-1. Studies are underway to determine whether or not the Ig or TCR genes are rearranged in this cells. Together, these findings indicate that the *in vitro* differentiation system parallels, to some extent, the onset of hematopoiesis within the developing embryo and as such, provides a valuable model for defining the molecular events involved in the earliest stages of hematopoietic development. SEND SEVEN additional copies

V-19 CHARACTERISATION OF A STEM CELL INHIBITOR: PRE-CLINICAL STUDIES
D. J. Dunlop, E. G. Wright[2], S. Lorimore[2], G. J. Graham[1], T. Holyoake[3], D. J. Kerr[1], S. D. Wolpe[4], and I. B. PRAGNELL[1]. [1]*CRC Beatson Laboratories, Glasgow, Scotland;* [2]*MRC Radiobiology Unit, Didcot, England;* [3]*Dept Haematology, Western Infirmary, Glasgow;* [4]*Genetics Institute, Cambridge, Mass 02140*

We have recently characterised a protein (murine macrophage inflammatory protein-1a) which is functionally, and antigenically identical to a stem cell activity in whole extracts of normal bone marrow[5]. Evidence will be presented that the human homologue (hu MIP-1a) inhibits proliferation of CFU-s *in vivo* and *in vitro* and that both stem cell inhibition and myeloprotection can be demonstrated *in vivo* in preclinical trials with a relevant therapeutic model suggesting an important physiological role for this protein in haemopoietic and other tissues.

[5]*Graham G J et al (1990) Nature 344, 442.*

V-20 K. Humphries

NO ABSTRACT SUBMITTED

V-21 Hepatic Cell Lineages. S. S. THORGEIRSSON. Laboratory of Experimental Carcinogenesis, National Cancer Institute, NIH, Bethesda, MD 20892.

Although the normal regenerative response of the liver following injury involves the replication of the existing hepatocytes, there are both experimental and pathological conditions that either inhibit and/or prevent this regenerative response. Under these conditions a facultative stem cell compartment is called upon to generate new hepatocytes and to rebuild the mass of the liver. The experimental models most frequently used to activate the stem cell compartment in rat liver involve pretreatment of the animals with low doses of 2-acetylaminofluorene that act as a mito-inhibitory agent for adult hepatocytes, prior to performing a partial hepatectomy. The new cell population that emerges following these experimental protocols had traditionally been referred to as oval cells due to the ovoid shape of their nuclei. It seems, however, likely that the cell population defined as oval cells represent a heterogeneous population containing subpopulation of cells with different developmental potential. We have recently demonstrated that oval cells can differentiate in vivo along the hepatocytic lineage and Evarts et al. (Carcinogenesis 8: 1737, 1987), Yaswen et al. (Am. J. Pathol. 121: 505, 1985, and Hayner et al. (Cancer Res. 44: 332, 1984) have shown that isolated oval cells contain two cell populations with isoenzyme patterns similar to either biliary duct cells or cells representing intermediate stages between biliary duct cells and hepatocytes. Furthermore, Hixon and Allison (Cancer Res. 45: 3750, 1985) have shown that oval cells may comprise a phenotypically complex set of cells composed of at least three antigenically distinct subpopulations. These and other recent data will be discussed.

V-22 Receptor Phenotype Underlies Differential Response of Hepatocytes and Non-parenchymal Cells to Heparin-Binding Fibroblast Growth Factor Type 1 (aFGF) and Type 2 (bFGF). W.L. MCKEEHAN, M. Kan, G. Yan, J. Xu, M. Nakahara, and J. Hou W. Alton Jones Cell Science Center, Inc., Old Barn Road, Lake Placid, NY 12946

Heparin-binding fibroblast growth factors (HBGF) have been implicated in the regeneration of both parenchymal and non-parenchymal cells of the liver. The response to and phenotype of hepatocyte receptors for HBGF-1(acidic fibroblast growth factor) and HBGF-2(basic fibroblast growth factor) was compared to keratinocytes, fibroblasts and endothelial cells. HBGF-1 stimulated DNA synthesis in hepatocytes, keratinocytes, fibroblasts and endothelial cells while activity of HBGF-2 was limited to fibroblasts and endothelial cells. HBGF-2 antagonized the mitogenic activity of HBGF-1 for hepatocytes and keratinocytes. Hepatocytes and keratinocytes exhibited both high- and low-affinity, non-matrix receptor sites for HBGF-1, but only low-affinity sites for HBGF-2. The mesenchymal cells displayed only high-affinity sites for both HBGF-1 and HBGF-2. Northern blot and immunochemical analysis revealed that the expression of HBGF receptor genes *bek* and *flg* are partitioned between normal hepatocytes and non-parenchymal cells, respectively. Expression of epithelial cell-specific, mesenchymal cell-derived HBGF-7, (keratinocyte growth factor) mRNA in regenerating liver tissue was undetectable relative to HBGF-1. The results support a multi-functional role of HBGF-1 acting through different receptor phenotypes in hepatocyte and non-parenchymal cells during liver regeneration.

V-23 BIOMATRIX AND LIVER CELL DIFFERENTIATION. D.M Bissell. Liver Center and Dept. of Medicine, University of California, San Francisco, CA 94110.

Recent work suggests that the Space of Disse in normal liver contains biologically active extra-cellular matrix (ECM). Experiments with liver cells in culture have shown that different substrata exert striking effects on specific gene expression by hepatocytes and lipocytes and on the morphology of sinusoidal endothelial cells. For all three cell types, substrata resembling a basement membrane are superior to interstitial collagen in supporting the normal state. This has led to the view that the ECM of the Space of Disse in normal liver may resemble a basement membrane, albeit a very low-density variant of the typical basement membrane. Supporting this view are data indicating that basement membrane proteins are present in or around the Space of Disse.

An alternative view emphasizes the classical definition of a basement membrane and concludes that the sinusoids lack this structure. As judged by standard transmission electron microscopy (TEM), an acellular lamina at the basal surface of an epithelium or at the abluminal surface of the sinusoidal endothelium is absent, at least in young rat liver, whereas a structure of this description appears in liver injury.

Clearly, a gap exists between biologists and morphologists on the subject of ECM in the Space of Disse. Because the biological data derive mainly from cells in culture, the potential for artifact exists. On the other hand, the morphological approach is limited by the ability of TEM to visualize low-density structures. What will bridge the gap is better information on the molecular details of basement membranes in normal and pathological states.

V-24

PLASMA MEMBRANE PROTEINS INVOLVED IN CELL-CELL CONTACT MEDIATED REGULATIONS OF HEPATOCYTE FUNCTIONS.

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Functional and morphological behavior of cells in a given tissue is strikingly controlled through various forms of intercellular communications involving both junctional and non junctional structures. In the liver, L-CAM is a major CAM early expressed in the fetal tissue and constantly maintained during the adult stage. It was identified as a component of *adherens junctions* and responsible for cell adhesion and polarity and for cytoskeletal organization *in vitro* as well as *in vivo*. Gap junctions defined as cell-to-cell channels, consist of aggregated hexameric structural units mainly formed by either a 26-27 kD protein (connexin 32) or a 21 kD protein. The amounts of the two proteins decrease and then increase similarly in primary cultures of fetal hepatocytes whereas the maximal coupling between adult cells has been related with their highest differentiated state. Interestingly, the 43 kD gap junction protein characteristic of myocardium has also been found in the liver and could be localized between hepatocytes and Ito cells in a coculture system. Among the non-junctional proteins, the cell CAM 105 defined as an ecto-ATPase, could participate in the modulation of hepatocyte adhesion. In contrast, the Liver Regulating Protein (LRP), recently identified in cocultures of hepatocytes with rat liver epithelial cells (RLEC) is not involved in cell adhesion or aggregation and is different from integrin molecules. It participates in coordinated regulations of liver-specific gene expression, cytoskeletal organization and extracellular matrix deposition. Recent data have evidenced its implication in differentiation of specific cell types from other tissues. This emphasizes the idea that the mechanisms by which cell-cell contacts may control cell functions could be, at least in part, common in several cellular systems.

V-25 Isolation of Differentiated Immortal Hepatocyte Lines from Transgenic Mice.
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Infection of fetal, newborn or adult mouse or rat hepatocytes in primary culture with SV40 virus results in their immortalization. Cells grow in response to EGF & insulin. Although lines retained hepatocate specific functions for 25 passages, their expression was not permanently stabilized. In contrast, immortal hepatocyte lines derived from transgenic mice bearing SV40 T-antigen-encoding constructs driven by the MT-I promoter in opposite orientation, stably express all tested liver specific functions in culture for at least 3 years. Lines retain the capacity to convert indirectly acting mutagens into genotoxic metabolites as shown by clastogenic activity & SCE induction by model compounds, and thus appear useful for short-term assay systems to identify potential mutagens. Cells are diploid and non-tumorigenic during the initial 40 passages, and acquire increasing growth autonomy from growth factors, cell-cell contacts & anchorage. In a later phase cells become aneuploid and finally gain the capacity to grow in agar & to form HCC upon injection into nude mice. Non-tumorigenic lines can be converted into transformed clones upon transfection with Hepatitis B virus (HBV) DNA, demonstrating that HBV display oncogenic potential in these cells.

V-26 Generation of Human Cytotoxic Lymphocytes Requires both IFN-γ and TNF-α-Mediated Signals.
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The role of physiologically secreted human IFN-γ in generation of MHC restricted CTL and LAK cells has been probed with a panel of mouse mAb against human IFN-γ or IFN-γR. Their addition to the culture medium inhibited proliferative and cytotoxic response to HLA alloantigens, conventional antigens and IL-2. The IFN-γ blockade also inhibited IFN-γ, IL-2 and TNF-α release during MLC. When IFN-γ was blocked, T-lymphocytes recovered from 6 days MLC displayed a profound decrease in their expression of p55 and p75 chains of the IL-2R. Antibodies to TNF-α, too, inhibited proliferation of alloactivated T-lymphocytes and generation of MHC specific CTL suggesting that the induction and the progression of lymphocyte activation is a stepwise process controlled by a cross-talk between lymphokines and their receptors. This possibility is further supported by the evidence of an almost parallel up-modulation of IFN-γR and IL-2R chains on $CD4^+$ and $CD8^+$ lymphocytes during the progressive phases of MLC. When the proliferative response peacks, p75 and p55 chains of IL-2R are still expressed, whereas IFN-γR is almost completely down-modulated.

V-27 **Stable Expression of Tumoricidal Activity by IL-2-dependent Human Leukemic T Cell Lines.**
D. SANTOLI and A. Cesano. The Wistar Institute of Anatomy and Biology, 3601 Spruce Street, Philadelphia, Pennsylvania 19104

We have established three human leukemic cell lines (TALL-103/2, TALL-104, and TALL-107) which express non-MHC-restricted cytotoxic activity upon culture in IL-2. Whereas TALL-103/2 cells ($CD3^+$ $TCR\gamma\delta^+$) kill only NK susceptible targets, TALL-104 and -107 cells ($CD3^+$ $TCR\alpha\beta^+$) are functionally comparable to LAK cells for their broad range of target reactivity and high killing efficiency. In reverse antibody-dependent cell-mediated cytotoxicity (ADCC) using the IgG Fc receptor-positive P815 target, lysis by all three cell lines is triggered by antibodies recognizing CD3 and CD2, but not CD8 or CD56 antigens. In conventional cytotoxic assays the lytic activity of these cell lines is strictly Ca^{2+}-dependent, whereas in reverse ADCC, lysis appears to only partially require extracellular Ca^{2+}. The cytoplasm of all three cell lines contains azurophilic granules typical of cytotoxic cells. Northern blot analysis demonstrates mRNA expression of pore-forming protein (PFP, perforin) and serine esterases (SE). Whereas TALL-103/2 cells express high levels of SE1 and low levels of PFP mRNA, the opposite is true for TALL-104 and -107 cells. The magnitude of lytic activity is dependent on the doses of IL-2. Upon deprivation of IL-2, TALL-103/2 cells completely lose cytotoxic granules and function within 16 h, whereas TALL-104 and -107 cells progressively lose expression of PFP and SE mRNA as well as killer activity within 4 wk. The cytotoxic efficiency of TALL-103/2 cells is highly and rapidly enhanced by several lymphokines including IL-4, IL-6, IFN-γ, and NKSF (IL-12). IL-12 increases also the lytic function of TALL-104 and -107 cells in synergy with IL-2.

The overall data indicate that the TALL-103/2, -104, and -107 cell lines express a highly regulatable tumoricidal activity, which renders them ideal for testing new adoptive immunotherapy strategies in suitable animal models.

V-28 Transfer of $CD8^+$ Cytomegalovirus (CMV)-Specific Immunity After Bone Marrrow Transplant By Adoptive Immunotherapy With T Cell Clones.
S.R. RIDDELL, K. Watanabe, J. Goodrich, C.R. Li, M. Agha, and P.D. Greenberg. Fred Hutchinson Cancer Research Ctr and Univ of Washington, Seattle, WA. 98104

Studies in our laboratory have demonstrated that the reconstitution of $CD8^+$ class I MHC restricted CMV-specific cytotoxic T cell (Tc) responses following allogeneic BMT correlates with protection from CMV disease. The protective immunodominant $CD8^+$ Tc response to CMV is specific for viral antigens introduced into the cytoplasm of infected cells immediately following viral penetration, resulting in target lysis prior to viral gene expression and new virion assembly. Therefore, we have isolated and propagated in vitro such CD8+ CMV-specific Tc clones from CMV seropositive marrow donors, and evaluated the potential for reconstituting immunity to CMV in the immunodeficient HLA identical recipient by adoptive T cell transfer during the period of high infectious risk. Five patients have been treated with 4 escalating weekly doses of CMV-specific Tc clones beginning 28-42 days posttransplant. All 20 T cell infusions were well tolerated with no alterations in vital signs or oximetry. The patients have been followed by functional analysis of CMV reactivity in PBL to determine the efficacy of T cell transfer in reconstituting immunity to CMV. Two patients with weak CMV-specific $CD8^+$ Tc responses prior to therapy had augmentation of these reponses following T cell transfer. Three patients had undetectable CMV-specific $CD8^+$ Tc and $CD4^+$ Th responses prior to T cell therapy--reconstitution of the CD8+ Tc (but not $CD4^+$ Th) response was demonstrable 2 days following infusion of the first dose of $CD8^+$ Tc clones (3.3×10^7 cells/m^2). Persistent $CD8^+$ Tc were detectable at the time of the next infusion, and the magnitude of the response was augmented with each subsequent infusion. Following completion of 4 infusions (total Tc dose $1.5 \times 10^9/m^2$), $CD8^+$ Tc responses persisted for >6 weeks. The persistence of transferred Tc was documented by demonstrating the TcR gene rearrangements present in CMV-reactive Tc clones derived from treated patients were identical to those employed in transferred clones. The results provide the first evidence in humans that adoptive transfer of antigen specific T cell clones can be used to reconstitute immunity in immunosuppressed hosts.

V-29 Molecular Events Mediating Activation of Cytotoxic Cells.

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The interaction of cytotoxic T lymphocytes with specific target cells produces the release of Ca 2+ from intracellular stores, and the transient formation of inositol trisphosphate (IP3), as well as the subsequent exocytosis of cytotoxic granules. Aspecific lysis by LAK cells is apparently independent of intracellular Ca 2+ rise, IP3 formation, and lytic granule release. These findings were confirmed when large granular lymphocytes (LGL) from patients with LGL lymphoproliferative disease were challenged with K562 susceptible targets. Interestingly, lysis of an antigen-specific CTL clone that acquired LAK-like activity following stimulation with supra-optimal doses of rIL-2 occured in the absence of second messenger formation and cytotoxic granule release. TCR signalling is also associated to tyrosine phosphorylation of a number of proteins; this phenomenon represents an early event that most likely precedes and is independent of IP3 formation and protein kinase C activation. Hence, we studied the effect of genistein, a tyrosine protein kinase (TPK) inhibitor, on cytotoxic cell activity. We observed that this agent inhibited or strongly reduced both CTL as well as LAK cell lytic activities. To evaluate tyrosine phosporylation of cytotoxic lymphocytes following interaction with susceptible targets in the presence of TPK inhibitors, immunoblotting studies are in course using anti-phosphotyrosine monoclonal antibodies.

V-30 H. Zur Hausen
West Germany

NO ABSTRACT SUBMITTED

V-31 Cell Cycle Regulation and Cellular Senescence. J.C. BARRETT, C.A. Afshari, L.A. Annab, B.A. Burkhart, J. Boyd, R.D. Owen, P.A. Futreal, G. Preston, and K.H. Richter. Lab. Molecular Carcinogenesis, NIEHS, Research Triangle Park, NC 27709.

We have proposed the hypothesis that cellular senescence is controlled by normal genes that are activated or whose functions become manifest at the end of the life span of a cell. Defects in the function of these genes can allow cells to escape the program of senescence and become immortal. This hypothesis is based on our ability to map senescence genes to specific chromosomes using somatic cell hybrids and microcell-mediated chromosome transfer. Candidate genes on human chromosomes 1, 4, and X have been mapped by these techniques, and we are currently attempting to clone a gene on chromosome 1q25. Senescent cells failed to phosphorylate the RB protein in response to serum. Since a central role for the $p34^{cdc2}$ protein kinase is postulated in control of the cell cycle and RB phosphorylation, we examined this kinase in senescent cells and observed no $p34^{cdc2}$ mRNA or protein. In other growth-inhibited states, brought about by isoleucine deficiency in G1, by aphidicolin at G1/S, by etoposide in G2, or by colcemid in M-phase of the cell cycle, cdc2 mRNA was expressed at high levels. Following transfection of a plasmid containing the human cdc2 gene into hamster cells, expression of cdc2 mRNA failed to overcome the block to DNA synthesis in senescent cells. Taken together, these data support the concept that a chain of events leads to senescence, where $p34^{cdc2}$ kinase is one of the critical elements but other cell cycle controls are also affected.

V-32 Transcription Factors, Proliferation and Cellular Aging in Human Fibroblasts. J. CAMPISI, Div. Cell & Molec. Bio., Lawrence Berkeley Lab., Bldg. 83, Univ. Calif., Berkeley, CA 94720

At the end of their proliferative lifespan in culture, human fibroblasts show changes in the expression of selected transcription factors. One change is repression of the c-fos protooncogene, a component of the AP-1 leucine zipper-type transcription complex. At early passage, c-fos is transiently induced early in G0/G1 and this is essential for proliferation. In senescent cells, c-fos is not induced by physiological mitogens, but it is induced by T antigen, the oncogene of SV-40 virus.

Microinjection of c-fos expression vectors restored Fos expression to senescent cells, but - in contrast to T antigen - failed to reactivate DNA synthesis. However, T antigen-driven DNA synthesis could be blocked by c-fos antisense oligonucleotides. This suggests that T antigen acts in part by derepressing c-fos, and that c-fos repression is an important, but not sole, event controlling the growth arrest associated with senescence.

A second factor is partially repressed in senescent cells: ID, which inhibits certain helix-loop-helix (HLH) transcription factors from binding DNA. ID is transiently induced early in G0/G1 and again late in G1. Both peaks of expression are partially repressed in senescent cells. A T antigen mutant (K1) that cannot bind the retinoblastoma tumor suppressor (Rb), does not reactivate DNA synthesis in senescent cells; however, overexpression of ID can partially restore the ability of the K1 mutant T to stimulate DNA synthesis. Thus, inactivation of Rb may be essential for the reactivation of DNA synthesis in senescent cells. ID may inactivate Rb directly, or growth suppression by Rb may involve an ID-sensitive HLH transcription factor.

V-33 Isolation of genes for cellular mortalizaton. S. SHALL, I.R.Kill, A. Broadhurst, & M. K. O'Farrell*. Cell and Molecular Biology Laboratory, University of Sussex, Brighton, Sussex; *Department of Biology, University of Essex, Colchester, Essex, ENGLAND.

The characteristic limited reproductive life-span of normal human fibroblasts in culture is due to a steadily decreasing fraction of cells able to proliferate in the standard rich growth media. We have previously postulated that there are genes (called mortalins), whose gene products induce mortalization (reproductive sterility or senescence) when their gene products reach a certain threshold (by accumulation or by dilution). Cell fusion experiments indicate that there are at least four complementation groups (genes), involved in regulating cellular mortalization, and also show that the genes act in a dominant fashion in the wild-type condition. We have begun a program to identify and clone the genes for cellular immortality. The procedure used is to inactivate the presumptive genes by insertional mutagenesis with DNA encoding a selectable marker in either plasmid DNA or in a retrovirus. Cells were then selected using the marker; and were then selected for immortalization. Over 20 independent cell clones have been isolated. One clone contains the inserted DNA in an open reading frame about 100 amino acids from a stop codon. This presumptive gene is not in the databases yet.

V-34 Role of Overexpressed Genes in the Replicative Senescence of Human Diploid Fibroblasts (HDF). S. GOLDSTEIN. Depts. of Medicine and Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences and GRECC, John L. McClellan Memorial Veterans' Hospital, Little Rock, AR 72205.

Because replicative senescence of HDF is a dominant trait, we have identified 18 overexpressed gene sequences from a cDNA library derived from senescent HDF mRNA (Murano, *et al.*, Mol. Cell. Biol. 11:3905-3914, 1991). Nine of these genes are known and include α1(I) and α2(I) procollagen, fibronectin, ferritin heavy chain, insulin-like growth factor binding protein-3 (IGFBP-3), osteonectin/SPARC, plasminogen activator inhibitor-type 1, thrombospondin, and αB-crystallin. The other nine sequences are unknown and include two novel genes plus seven with both novel segments and the Alu (SINE) and KpnI (LINE-1) families of repetitive elements. IGFBP-3 mRNA levels are constitutively high in senescent compared to young HDF, and the cognate protein accumulates to high levels in senescent HDF-conditioned medium, so that the molar ratio of IGFBP-3/IGF-I + II readily exceeds 1. Moreover, IGFBP-3/IGF-I molar ratios ≥ 1 are associated with extinction of IGF-I-stimulated DNA synthesis. The sequence of one hitherto unknown cDNA appears to encode the human homolog of a 22kDa chicken smooth muscle protein; it fails to inhibit DNA synthesis but may affect other properties of senescent HDF. Concerted overexpression of IGFBP-3 and many of these other genes may contribute to replicative senescence of HDF, diminished tissue repair in aged persons, and age-dependent degenerative disease. Conversely, stepwise loss-of- function mutations in one or more such genes may play a role in cellular immortalization, tumorigenesis and metastasis. Supported by grants from the NIH (AG08780), NSF and the Department of Veterans Affairs.

V-35 Senescent Cell Derived Inhibitors of DNA Synthesis. A. Noda, S. Venable and J.R. SMITH. Division of Molecular Virology, Baylor College of Medicine, Houston, TX 77030.

In vitro cellular senescence of human fibroblast-like cells is characterized by the irreversible loss of DNA synthesis ability. Somatic cell hybridization studies have shown that the phenotype of in vitro cellular aging is dominant over the phenotype of cellular immortality. Other studies have shown that senescent cells produce an inhibitor of DNA synthesis. The inhibitor that has been previously extracted from senescent cells appears to be a plasma member associated protein encoded by a highly abundant mRNA. Direct functional screening of a cDNA library produced from senescent human fibroblasts identified three distinct cDNA clones that inhibited DNA synthesis when transfected into young human fibroblasts. Two of the clones hybridized with mRNA's that were of approximately the same relative abundance in senescent and young cells. One clone, called SDI-1, hybridizes with a 2.1Kb poly(A)$^+$ RNA which increases more than 10 fold during cellular aging. At the present time, the casual relationship between increased expression of SDI-1 and cellular senescence is not known.

V-36 Wilson H. Burgess
American Red Cross, USA

Structure-Function Studies of FGF-1

NO ABSTRACT SUBMITTED

V-37 Jeanette A. M. Maier
University of Brescia, Italy

Cellular Signals Controlling Endothelial Cell Proliferation and Gene Expression

NO ABSTRACT SUBMITTED

V-38 Venkat Gopal
Otsuka Pharmaceuticals, USA

Gene Transfer in Human Endothelial Cells In Vitro

NO ABSTRACT SUBMITTED

V-39 David Shepro
Boston University, USA

NO ABSTRACT SUBMITTED

W-1 Use of a Cell-Free Medium to Determine the Source of a Parasitism-Specific Protein Found in the Hemolymph of a Fruit Fly Host. PAULINE O. LAWRENCE. Department of Zoology, 223 Bartram Hall, University of Florida, Gainesville, FL 32611.

A 24 kD protein was discovered in the hemolymph of pharate pupae of the Caribbean fruit fly Anastrepha suspensa after parasitization of larvae by the wasp Biosteres longicaudatus. To investigate the possible source of the 24 kD protein, first instars, their extra-embryonic membranes (serosas) and 2nd through 4th (final) instars of B. longicaudatus were incubated for 3-5 days at 25 ± 2°C and 98% R.H., in Goodwin's IPL 52B supplemented with 20% fetal calf serum (FCS). At the end of the incubation period, the culture media and homogenates of their respective larvae were subjected to immunodetection (Western blotting) with a rabbit polyclonal antibody generated against the 24 kD protein purified from A. suspensa hemolymph. The protein was detected in homogenates of the serosa and in all parasite instars as well as in their culture media. Glycoprotein staining of the media and host hemolymph indicated a high mannose content of the 24 kD protein. The protein was also detected in Western blots of host hemolymph as early as 4 h post parasitization when the parasite was still in the egg stage. The in vitro results suggest that the protein is of parasite origin and is released into the host's hemolymph in vivo. However, its early appearance during the parasite's egg stage suggests that it may be introduced into the host at oviposition, when the female wasp is known to inject an entomopoxvirus which is incorporated in the parasite. Thus, viral induced synthesis of the 24 kD protein by parasite larva cells may occur.

W-2 Embryonic Development of an Endoparasitoid, Microplitis croceipes (Hymenoptera: Braconidae) In Vitro. S. FERKOVICH and H. Oberlander. USDA/ARS, P.O. Box 14565, Gainesville, Florida 32604

The endoparasitoid, *Microplitis croceipes*, is an important parasite of the bollworm, *Helicoverpa zea* and the tobacco budworm, *Heliothis virescens* in the southern region of the U.S. Because of its potential use as a biological control agent, development of an artificial culture medium to replace the host would facilitate mass rearing of the parasitoid. We have been successful in promoting embryonic development of the parasitoid from pregerm band stage to first larval instar in cell culture medium conditioned by a cell line (IPL-LdFB) derived from fat body from an atypical host, *Lymantria dispar*. However, the percentage of eggs normally developing to the first larval instar stage is significantly less that those maintained in IPL-52B medium conditioned with host fat body tissue. In addition, earlier studies indicated that IPL-52B medium conditioned by the imaginal disc cell line, IAL-TND1, promoted germ band formation but did not stimulate hatching. In this study, we examined the capacity of one embryonic, and six tissue-derived cell lines to promote growth and development of pregerm band eggs in three media (IPL-52B, TC 199-MK, TC 100, Grace's, and EX CELL 400). The developmental response of *M. croceipes* was dependent both on the nature of the cell line and the cell culture medium used. For example, BCIRL-HV did not induce hatch in IPL-52B medium but did promote it in TC 199-MK medium. BCIRL-HZ-AM1 stimulated hatch only in TC 199 MK while IAL-TND1 induced hatch both in IPL-52B and Grace's medium. Thus, the composition of the medium, and the species and tissue type of the cell line source must be evaluated interactively to determine optimal conditions for promoting development of *M. croceipes* in vitro.

W-3 Infection of Choristoneura fumiferana (Lepidoptera: Tortricidae) Cell Lines with the Microsporidium Pleistophora schubergi Zwolfer. S.S. SOHI, and G.G. Wilson, Forest Pest Management Institute, Forestry Canada, P.O. Box 490, Sault Ste. Marie, Ontario P6A 5M7, Canada.

A cell line, FPMI-CF-34 (CF-34), was developed from ovarian tissues of the spruce budworm Choristoneura fumiferana pupae that were naturally infected with the microsporidium Pleistophora schubergi. This infection was carried over into the cell cultures. A second cell line, IPRI-CF-1 (CF-1), developed from healthy neonate larvae of C. fumiferana was successfully infected with the microsporidium by introducing medium or cells from the infected CF-34 culture. Initially, CF-1 cultures inoculated with CF-34 cells infected with P. schubergi had higher infection than the cultures inoculated with infected culture medium. After about 5 months, however, the level of infection in both groups was comparable. A reduction in cell growth occurred in the infected cultures. P. schubergi spores produced in cell cultures were infectious for C. fumiferana larvae. This microsporidium has a wide host range, infecting several forest insect defoliators, and the results from this study would indicate that it can be propagated in cell culture.

W-4 Gene Expression of a Baculovirus Recombinant in Permissive and Nonpermissive Insect Cells. A.H. McINTOSH and J.J. GRASELA, USDA, ARS, Biological Control of Insects Research Lab, P.O. Box 7269, Columbia, MO 65205.

Reporter genes encoding enzymes with readily detectable activities have found widespread use in applied and fundamental biology. An AcMNPV recombinant (Ac-Gal-Luc) carrying the β-galactosidase and luciferase genes under the control of p10 and polyhedrin promoters, respectively was used to assess expression in permissive and nonpermissive insect cell lines. Lepidopteran cell lines employed included Trichoplusia ni (Tn-CL1), Spodoptera frugiperda (IPLB-Sf21), Plutella xylostella (BCIRL-Px2-HNV3), Helicoverpa zea (BCIRL-HzAM1), H. armigera (BCIRL-HaAM1), Heliothis virescens (BCIRL-HvAM1), H. subflexa (BCIRL-HsAMI), and a coleopteran cell line (AGE) from Anthonomus grandis. Cells were inoculated with Ac-Gal-Luc at an MOI of 10 and incubated at 28°C for 72 hr following removal of inoculum, washing of cells with CMF-PBS and addition of 5 ml TC 199-MK to each T25 cm^2 flask. At the end of this period both supernatant fluids and cell lysates were assayed for enzyme activities employing commercially available kits. β-galactosidase was detectable in both supernatant and pellets from Tn, Px, Hv, Hs, Sf and AGE cells. Activities in permissive cell lines ranged from 1.1 X 10^6 - 2.9 X 10^6 pg/ml. Enzyme activity was marginal for Hz and Ha cells. Luciferase activity could be detected at a concentration of 1 X 10^{-12} g/ml in Px, Sf, Tn, Hs and AGE cell lines.

W-5 Plant germplasm storage technology - workshop. P.C. Stanwood. USDA-ARS National Seed Storage Laboratory, Colorado State University, Fort Collins, CO 80523

Long-term preservation is an integral and necessary activity to the management and utilization of plant genetic resources. Utilization of these resources can be viewed as a continuous process starting with plant (gene) exploration continuing into evaluation, increase, distribution then ending with utilization. Long-term preservation technologies provide support for these processes. Additionally, the resources are conserved, building a gene library for future generations of man. This workshop will address preservation technology issues under four main topics: 1) Plant germplasm preservation management systems; 2) storage of 'orthodox' and 'recalcitrant' seeded species; 3) conventional clonal material preservation; and 4) cryopreservation. A discussion of the U.S. National Plant Germplasm System (NPGS) will open the workshop followed by individual storage technology issues. The core of the NPGS is based around four regional plant introduction stations, nine national clonal germplasm repositories, approximately ten crop-specific collections, the U.S. National Arboretum, the Germplasm Resources Information Network, the National Germplasm Resources Laboratory, the National Plant Germplasm Quarantine Center and the National Seed Storage Laboratory. There are approximately 400,000 accessions in the system presenting 3,500 species with 10,000 new accessions added yearly. The system fills 2,000 requests for material each year.

W-6 Techniques for Clonal Germplasm Preservation. BARBARA M. REED USDA/ARS National Plant Germplasm Repository, 33447 Peoria Road, Corvallis, Oregon 97333

Storage of genetic material of clonally propagated plants has become increasingly important in recent decades to provide materials for development of new cultivars as well as preserving heirloom varieties and rare or endangered species. In some cases plants may be lost before habitat can be protected. Conventional storage includes growing plants in the field, greenhouses or screenhouses, or as in vitro cultures. Field collections face problems with disease, environmental and insect damage, space, labor and lack of monetary resources. Clonal material must be evaluated for potentially important traits and the types and quantity of plants to be stored and the proper sites for preservation must be determined. Materials should be tested for viruses and related organisms and protected from disease and insect pests which might affect the survival of the plant or be spread by distribution of germplasm to previously uninfested countries. In vitro culture is an efficient technique that satisfies these storage and quarantine requirements. It also provides a backup for field collections. Many genera can be stored as tissue cultures for extended periods at reduced temperatures. Genetic change in clonal and in vitro plants is genotype dependent and must be examined individually. With proper care, clonal germplasm could be preserved indefinitely and should enable gene banks to supply many future generations of scientists and breeders.

W-7 Cryopreservation and other technologies for long-term storage of clonal crop germplasm. L.E. TOWILL. USDA-ARS National Seed Storage Laboratory, Colorado State University, Fort Collins, CO 80523

Effective germplasm preservation utilizes both active and base collections. The latter comprises accessions held under minimal maintenance and serves as a backup to materials held in the active collection. Cryopreservation, as a low-maintenance, long-term storage system, is more useful for base collections, especially for those of species held as clones. Shoot tips or buds are used for cryopreservation and are obtained from either in vivo or in vitro plants. The method of choice depends on the physiological state of the parent material. Dormant buds are used for cold hardy species. Partial desiccation and slow cooling are applied; retrieval after storage is by grafting. Shoot tips are isolated from non-cold hardy species and are exposed to cryoprotectants prior to cooling. Retrieval after storage involves culture of the shoot tip; direct development of the shoot tip into a plant is desired to minimize selection of variants. Two-step cooling and vitrification protocols have been demonstrated to preserve shoot tips/buds from both hardy and non-hardy species after liquid nitrogen exposure. Variations of both protocols exist, including pretreatment of stock plants and shoot tips, cryoprotectant concentrations and rates of addition and removal, cooling and warming rates, and post-treatment recovery procedures. Although several problems remain for effective utilization, cryogenic storage has become feasible and will play a role in germplasm preservation in the near future. Other technologies will provide supplemental methods for long-term preservation. For example, somatic embryogenesis and nucleic acid characterizations and manipulations are rapidly advancing such that use for some aspects of germplasm preservation may occur in the future.

W-8 The Use of Tissue Culture Techniques in the Preservation of 'Orthodox' and 'Recalcitrant' Seeds. H W PRITCHARD. Jodrell Laboratory, Royal Botanic Gardens, Kew, Wakehurst Place, Ardingly, West Sussex RH17 6TN, UK

The storage physiology of seeds can be essentially divided into two broad categories: desiccation tolerant or 'orthodox' and desiccation intolerant or 'recalcitrant' seeds. A majority of 'orthodox' seed can be preserved long term at sub-zero temperatures and a moisture content of about 5% (f.wt basis), with viability assessment on water and an inert substrate such as agar or filter paper. However, there are numerous situations where the use of tissue culture techniques are essential to the assessment of or the recovery of viability after seed storage. Firstly, some seeds with an undifferentiated embryo will only germinate when nurtured in vitro: in the orchids dixenic culturing with a symbiotic fungus may be required. Secondly, embryo culture can circumvent the widespread problem of seed dormancy in wild species to provide a rapid estimate of viability. Finally, embryo rescue techniques can be applied to recover viability from aged seeds. In contrast to 'orthodox' seed, embryonic axes of 'recalcitrant' seed are inviable when dried to c.20% moisture content within the seed, and thus seed conservation at low moisture contents is not possible. However, isolated axes of at least seven species can exhibit some organised growth in vitro after rapid drying to $\leq$17% moisture content. The ability of these dried axes to survive exposure to sub-zero temperatures, combined with the potential for rapid clonal propagation from axillary buds at the base of the cotyledonary petioles, forms the basis of an in vitro conservation system for 'recalcitrant' seed material.

W-9 Time-Lapse Cinematography of Hemocytes of the Clam, Mercenaria mercenaria. A.F. EBLE.Trenton State College,Dept.Biology Trenton, NJ 08650-4700.

Clams were collected at Shark River, Belmar, N.J. and maintained in all-glass aquaria with filters; salinity was 28‰ and temperature 22°C. Blood was removed from the anterior adductor muscle and placed on No. 1 cover slips. Cell activity was viewed with Zeiss Photomicroscope II fitted with a Sage time-lapse system using a Bolex camera with 16mm Kodak Plus X reversal film. Both phase contrast and Nomarski interference phase optics were employed.Events were speeded up 35 times.Granulocytes had a large endoplasm filled with granules that were a mixture of lysosomes, secondary lysosomes,lipid droplets and mitochondria; ectoplasm was quite thin and formed many filopodia. Very active locomotion was observed with much membrane ruffling. Movement of granules in the endoplasm around the centrosome was very obvious. Hyalinocytes were larger than granulocytes, had a small endoplasm with few granules and a large ectoplasm. This cell type exhibited no motion when viewed in real time but time-lapse cinematography revealed a limited locomotion with moderately intense membrane ruffling. Granulocytes readily phagocytosed boiled yeast cells;internalization was rapid and phagosomes of 4-8 yeast cells were not uncommon. Hyalinocytes were not active in phagocytosis.

W-10 **Influences of the Oyster Environment on the Activities of Oyster Hemocytes in Primary Culture. W.S. FISHER. U.S. Environmental Protection Agency, Gulf Breeze ERL, Sabine Island, Gulf Breeze FL 32561.**

Oysters (Crassostrea virginica) are poikilothermic and osmoconforming, so their hemocytes are exposed to the same thermal and salinity changes that occur in the estuarine and marine environment. In vitro assays were developed to study the potential influences of these changes on hemocyte activities. Initial studies demonstrated that hemocytes in primary culture were affected by acute changes in salinity; change to a hyperosmotic salinity retarded the ability of hemocytes to spread to an ameboid shape and slowed their rate of locomotion. Further application of these assays to laboratory and field studies revealed that in vitro hemocyte activities were influenced by the environments in which the oysters lived. Hemocytes from oysters of low-salinity origin exhibited reduced rates of locomotion even after one month in high salinity conditions. Hemocytes from oysters held at high temperatures (25°C) exhibited slower locomotion than hemocytes from oysters held at low temperature (15°C). Salinity and temperature effects on rate of locomotion were additive. Field collections of oysters from different habitats at different times of year supported laboratory findings of the influence of temperature and habitat: A marked decrease in hemocyte activity occurred during the hot summer months, and the effect was different for estuarine and marine oysters.

W-11 In Vitro Interactions between Molluscan Hemocytes and the Parasitic Protozoan Haplosporidium nelsoni (MSX) S.E. FORD and K.A. Alcox. Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ 08349.

The parasitic protozoan Haplosporidium nelsoni, cause of MSX disease, is highly lethal to its host, the eastern oyster, Crassostrea virginica. Previous research using tissue sections, and preliminary in vitro observation, indicated that oyster hemocytes do not phagocytose the parasite. To further study cellular responses to H. nelsoni, we developed an in vitro assay that allows us to manipulate and quantify hemocyte-parasite interactions. Parasites are collected from infected oysters, enriched by panning, and incubated with hemocytes. Either host or parasite may be experimentally manipulated prior to incubation. Microscopic observations then determines whether parasites have been ingested, have adhering hemocytes, or are solitary. This assay has verified that phagocytosis of H. nelsoni by oyster hemocytes is very low (<5%), even in strains that are resistant to MSX disease, indicating that phagocytosis of parasites, in this case, is not a defense mechanism. Killing of parasites by low-salinity immersion, however, resulted in high phagocytic rates (>95%). Experimental treatment of parasites with general, but not specific, proteases significantly enhanced phagocytic rates; however, carbohydrases did not affect phagocytosis. Parasites treated with metabolic inhibitors were phagocytosed at significantly higher rates than were controls. Results of these experiments suggest that parasite surface structure may prevent recognition and consequent failure of oyster hemocytes to phagocytose H. nelsoni. The increase in phagocytosis after metabolic blocking, however, may also indicate that some type of inhibitory molecule is produced by parasites.
NJAES Publication No. 32405-1-92.

W-12 Infection of Oysters with *Perkinsus marinus* Enhances Chemiluminescence by Hemocytes. R.S. ANDERSON[1], K.T.Paynter[2] and E.M. Burreson[3]. [1]Chesapeake Biological Laboratory, University of Maryland System, Solomons, MD 20688, [2]Department of Zoology, University of Maryland, College Park, MD 20742, [3]Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Hemocytes from the Eastern oyster, *Crassostrea virginica*, produce luminol augmented chemiluminescence (CL), particularly after phagocytic stimulation. This activity is generally attributed to the production of reactive oxygen species that are involved in phagocyte-mediated defensive mechanisms such as bactericidal and other cytotoxic responses. Therefore, CL measurements find use as indicators of nonspecific immunological capacity of blood cells in normal and stressed animals. The protozoan *P. marinus* causes one of the major diseases of oysters; it exists free in the hemolymph, as well as within the hemocytes. In this study, the CL response was quantified in hemocytes withdrawn from oysters with known intensities of *P. marinus* infection. The animals were transplanted to a site of high infection pressure, periodically sampled, hemocyte CL activity assessed by single photon monitoring, and individually diagnosed and scored for infection level. Parameters such as the phagocytically-induced CL peak response value and the total area under the induced CL curve were significantly higher in cells from oysters with advanced *P. marinus* infections. Not only was the per cell oxyradical production enhanced, but also the actual number of circulating hemocytes were increased concomitant with disease progression. These changes may result from in vivo hemocytic activation and/or cell recruitment caused by the protozoan parasites; the resultant increase in oxidative damage probably contributes to the pathology of the disease. (Supported in part by NOAA Grant NA16FL0400-01).

W-13 Survival and Changes in Pharyngeal Explants of a Solitary Tunicate. T. Sawada[1], E.L. COOPER[2], J. Zhang[2]. [1]Department of Anatomy, Yamaguchi University, Ube, Japan 755. [2]Department of Anatomy and Cell Biology, School of Medicine, University of California Los Angeles, Los Angeles, CA 90024.

Tunicates are fully capable of showing inflammatory reactions as well as specific cellular and humoral responses to antigens that may be pathogenic. A more thorough elucidation of these responses has been hindered by the lack of a reliable culture system for tunicate tissue. These culture techniques are essential in developing in vitro assays which could resemble mixed leukocyte reactions or cytotoxic responses. We cultured pharyngeal explants for 81 days. In the culture explants shrank, many free cells migrated from them into the culture medium, and the remaining hemocytes inside the explants became sparse. Autoradiography with 3H-thymidine incorporation showed that cell proliferation in hemocytes still occurred within pharynx cultured for three weeks. Cells leaving the explants into culture medium were counted and examined by neutral red-staining in the living state and by hematoxylin-eosin staining after fixation with paraformaldehyde. The numbers of cells increased to the maximum at 16-24 days (4.3×10^5 / explant) and decreased to 4.9×10^4 at 74-81 days. Many of these cells were not like hemocytes found in normal hemolymph. Many encapsulations containing 3-8 cells were also observed. Transmission electron microscopy of hemocytes remaining within the explants revealed changes in hemocyte composition; hemoblast-like cells increased shortly after the start of culture (8.1% at 0 days increased up to maximum 28.7% at 7th day). This study confirms a previous one with respect to viability and proliferation. We are now certain of a reproducible technique for culturing tunicate tissues which will aid in developing assays for immunologic research. Supported by DCB90-05061 from NSF.

W-14 The Inducible Humoral Immune Response in the American Cockroach: Site of Synthesis of the Immune Proteins. R. D. KARP and S.Ewashinka. Dept. of Biol. Sci., Univ. of Cincinnati, Cincinnati, OH 45221.

Previous studies established that the American cockroach generates true adaptive humoral immunity when immunized with soluble proteins. SDS-PAGE analysis of female immune hemolymph consistently shows an increased production of proteins with M_rs of 95kD, 102kD and 115kD. Studies indicate that the 102kD protein appears to be the most significantly related to the immune state. When immune hemolymph was analyzed using non-reducing PAGE, two of the bands were consistently enhanced. These bands specifically bind to the inducing antigen. If the antigen is toxic in nature, the induced bands can be eluted from gels, and used to passively protect naive animals against the antigen's lethal effects. When the non-reduced protein bands were subjected to SDS-PAGE, they were found to be the source of the 102kD protein. In order to determine the source of these immune proteins, we have initiated de novo studies to see if immune proteins are made in the fat body, which is a major source of protein in the insect, and/or circulating hemocytes. Female roaches were injected with either cytochrome c or sterile saline. Animals were bled for hemocytes and then dissected to remove the fat body. Hemocytes and fat body were placed in S-20 culture medium (Gibco) with 10% FCS, 1% antibiotic solution and 1% glutamine. Each tissue was exposed to the antigen during the culture period. ^{35}S-Met, Cys label was added immediately upon culture set-up. Supernatants were collected after various periods of time, and subjected to SDS-PAGE analysis and fluorography. Initial results indicate that both tissues were actively synthesizing proteins in culture, but only hemocytes generated the 102kD protein. Supported by NIH research grant GM 39398.

W-15 Low Cost Air Lift Bioreactors for Multiple Comparisons of Growth Conditions. NORMA L. TROLINDER, James Parker, Randy D. Allen, and Chris Ashcroft. USDA-ARS, Route 3, Box 215, Lubbock, TX 79401 and Texas Tech University, Chemical Engineering, Lubbock, TX 79409.

The high cost of equipment hampers the ability to empirically determine the optimum medium and growth conditions for plant cell growth and differentiation in bioreactors. A multidisciplinary research team will discuss and demonstrate the manipulation of multiple low cost air lift bioreactors that has been instrumental in optimizing growth conditions for cotton cells. Problems and solutions for continuous feed, batch, and semi-batch modes will be investigated.

W-16 ***THE MEDIA ARE NOT WHAT THEY SEEM:*** *photochemical changes in the composition of plant tissue culture media*

Roger P. Hangarter, Plant Biology, Ohio State University, Columbus, Ohio 43210

When plant tissue culture medium is exposed to light from fluorescent bulbs, EDTA is oxidized by photochemical reduction of ferric iron. Products of the oxidized EDTA include formaldehyde which can accumulate to inhibitory levels. Furthermore, the medium becomes iron deficient since unchelated iron forms an unavailable precipitate as EDTA is oxidized. The combination of light-induced formaldehyde production, and loss of available iron reduces the ability of the culture media to support tissue growth. Furthermore, growth regulators such as indole-3-acetic acid are also degraded by iron-catalyzed photochemistry, and such photodegradation results in reduced growth of tissues that require exogenous growth regulators. Removing UV and blue wavelengths with a yellow acrylic filter is a simple and effective means of preventing light-induced Fe-catalyzed alterations in plant culture media.

W-17 ENVIRONMENTAL EFFECT AND ENVIRONMENTAL CONTROL IN PLANT TISSUE CULTURE

T. KOZAI and K. Fujiwara
Faculty of Horticulture, Chiba Univ.,Matsudo, Chiba 271, Japan

In this presentation, the authors describe: (1) important physical environmental factors in plant tissue culture, (2) the features of physical environment in plant tissue culture, (3) environmental effects on the carbon balance, growth and development of shoots/plantlets in vitro under heterotrophic, photomixotrophic and photoautotrophic conditions, (4) measurements and control of the physical environmental factors.

Physical environmental factors discussed here are light, carbon dioxide, humidity, temperature, oxygen, air flow speed, etc.

Practical applications of the environmental control for promoting the photosynthetic growth, controlling the plant height (shoot length), reducing the vitrification, reducing the lighting and cooling costs, and saving the space required for placing the culture vessels are demonstrated.

Special attention is given to the sideward lighting using diffusive optical fibers, application of the DIF (Difference in air temperature between photoperiod and dark period) theory to control the shoot length, and carbon dioxide enrichment under photoautotrophic conditions for promoting the photosynthetic growth.

W-18 **Complex Effects of Physical Support on In Vitro Growth.** M.A.L. SMITH and L.A. Spomer, Univ. of Illinois, Urbana, IL 61801

Apparently minor changes in the physical support for plants growing in vitro can actually affect dramatic changes in plant tissue quality, proliferation rate, elongation, or other response. Liquid culture enrichment (periodic liquid medium overlays on a solid agar base) enhances miniature rose growth, as compared to gel-solidified culture, independent of subculture frequency or medium volume. The same explants become vitrified in liquid culture, unless dried at the time of subculture (to increase surface tension) and floated on the liquid surface. Similar in vitro growth responses are traced back to differences in gelling agent, gel concentration, or duration of medium storage prior to use. While well-recognized, and often deliberately exploited by researchers to optimize in vitro production, the underlying physical cause for the physiological effects has been difficult to establish. The type of physical support seems to exert a powerful influence on the water (and dissolved nutrients) available to explants, which has been hypothesized as water potential/water availability/gel matric effects. Conventional water measurements (thermocouple psychrometer [osmometer] and suction plate), however, don't support the hypothesis that gel effects are due exclusively to water availability. Comparative measurements from new, direct tests (on water availability, water flux, and gel matric potential) will be presented to establish water relations of gel media and to elucidate the underlying basis for microculture responses to different physical support conditions.

W-19 Expression of Par Genes during the Transition from G_0 to S Phase in Tobacco Mesophyll Protoplasts.

T. NAGATA and Y. Takahashi, Department of Biology, Faculty of Science, University of Tokyo, Hongo, Bunkyo-ky, Tokyo 113, Japan

The cell cycle transition from G_0 to S phase has been pursued with a specific stress on the role of auxin in tobacco mesophyll protoplasts cultured in vitro, as the differentiated and non-dividing tobacco mesophyll protoplasts gain meristematic activity upon culture on the medium containing auxin and cytokinin. We could isolate 3 auxin-regulated genes by differential screening regarding auxin; i.e. par (protoplast auxin-regulated)A, parB and parC. These genes expressed in fact during the transition from G_0 to S phase, when we examined their expressions by Northern hybridization. ParA and parC are suggested have a role in the transcriptional regulation from their homology to the bacterial stringent starvation protein, while parB encodes glutathione S-transferase. The role of these gene products is discussed in relationship to the cell cycle transition. Furthermore, we have identified auxin-responsive cis-acting regions in the 5' non-coding region of parA gene and an auxin-binding protein (ABP) of 22kD in the membrane fraction of tobacco mesophyll protoplasts which is immunologically identical with the well characterized maize ABP of 21 kD. Thus the chain from auxin application via gene expression to cell cycle progression will be discussed.

W-20 Synchronized Plant Cell Cultures as a Means to Study the Temporal Linkage of Cell Cycle Events.

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The investigation of the regulation of the cell cycle largely depends on the availability of efficient synchronization protocols. Using flow cytometry we have tested and optimized a method employing aphidicolin as a synchronizing agent. Inhibition of DNA replication and subsequent release from the blockade resulted in a synchronous progression of cells through S, G2, and M phase in a high proportion (up to 70%) of the total cell population. Comparison with phase duration data obtained from asynchronously dividing cells revealed that synchronous cell cycle progression after aphidicolin treatment closely resembles the asynchronous cell cycle and shows no alterations in the phase durations. The G1 phase appears to differ to a great extent as a second round of replication yields a much smaller and broadened peak of S phase cells in the flow cytometric histograms indicating that entry into S phase is delayed. Treatment with aphidicolin therefore appears to be useful for synchronization of plant cells during S, G2, and M phase of the first cell cycle but not for G1. We have tested the feasability of the method by assaying the expression and activity of major G2-specific control factors (plant cdc2 / cyclin B) throughout the synchronized cell cycle.

W-21 Cell Cycle Initiation and Progression in Cultured Protoplasts derived from Differentiating and Differentiated Plant Cells

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Differentiated plant cells are usually considered to be totipotent entities, because cell division and plant regeneration can be obtained from isolated, differentiated cells or protoplasts in a number of species, e.g. in Nicotiana. Yet, not all species behave according to the rules'. In the cereals, for instance, differentiated cells have proved to be extremely recalcitrant, even to reenter the mitotic cycle.
We have isolated and cultured mesophyll protoplasts from leaf tissues at different stages of development in Nicotiana and in cereals, including oats and wheat. Potential cell cycle initiation and progression was analysed by various means.
In Nicotiana cell division could be obtained in protoplasts derived from leaf stages ranging from very immature, just expanding to fully differentiated, almost senescing leaves. However, a decreasing sensitivity towards auxin was observed with increasing age i.e. differentiation of the leaves, spanning several orders of magnitude of auxin concentrations. Suboptimal auxin levels resulted e.g. in precocious cell expansion and incomplete cell cycles.
In the cereal protoplasts cell cycle progression and coordination was sluggish and eventually halted at different phases, depending on the stage of donor tissue differentiation and on the species tested. The behaviour resembled that of Nicotiana protoplasts inadequately supplied with auxin, even in protoplasts derived from immature, expanding leaf tissue. The implications for cereal protoplast culture in particular and plant tissue culture in general will be discussed.

W-22 COMPONENTS OF THE CELL CYCLE CONTROL IN CULTURED PLANT CELLS

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Division and differentiation of plant cells in cultures is highly dependent on external hormone effects, that is responsible for activation of signal transduction system involved in cell cycle control. Based on immunological detection system we provide evidences for the presence of $p34^{cdc2}$ kinase and cyclin A in protein fraction bound to the $p13^{suc1}$ sepharose. In synchronized alfalfa cells the $p34^{cdc2}$ kinase activity showed variation during the cycle with a maximum at S-phase. Similar conclusion can be drawn from studies on cell cycle activation in leaf protoplast cultures grown in the presence of auxin. Characteristic transcriptional changes can be detected by Northern analysis of RNA samples from cells at various cell cycle stages. Differential expression of histone H3 variants, cdc2 and other kinase or auxin responsive genes can be correlated with reprogramming of somatic cells during the course of mitotic reactivation or cell cycle progression. The S-phase dependent expression of histone H3 promoter was proven by analysis of GUS reporter gene in synchronized alfalfa cells or transgenic tobacco plants.

W-23 Gene Expression during the Cell Cycle in the Synchronized Cultures of Catharanthus roseus Cells.

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We characterized the gene expression during the cell cycle in higher plant cells. Cells of Catharanthus roseus grown in suspension can be synchronized by two different methods. In one system, synchronous cell division is induced by double phosphate-starvation method, and in the other, it is induced by auxin starvation method. Using these two systems, we isolated the cDNAs which were expressed in the specific phases of the cell cycle. One of the isolated cDNAs, named as cyc07, was characterized in detail. cyc07 gene encodes a 1.2-kb mRNA, which appeared preferentially in the S phase during the cell cycle in both synchronous cell division systems. The expression of cyc07 was closely associated with cell proliferation in both intact plants and cultured cells. We found that the yeast contained two closely related genes which were homologous to cyc07. These yeast genes were named as PLC1 and PLC2. Both of PLC1 and PLC2 genes were expressed specifically in the rapidly proliferating cells of yeast. Site-directed disruption mutations were produced in both genes. Cells with both mutations could not grow. The rate of cell proliferation varied with the gene copy number. Thus PLC1 and PLC2 constitute an essential gene family for proliferation of yeast cells. These results suggest that the gene cyc07 encodes a protein which plays an important role in the progression of the cell cycle in higher plant cells.

W-24 Engineering Experiences with Bioreactor Scale-Up of Somatic Embryogenesis

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A bioreactor culture system is commmonly considered when discussing scale-up and automation of somatic embryo production. However, successful culture protocols developed for shaker flask embryo production often are ineffective when applied to the bioreactors culture environment. This workshop will examine some of the significant differences between these culture environments including areation, agitation, and culture densities. An overview of plant tissue culture bioreactor technology, both commercial and experimental, will be presented. This will include a description of a bioreactor system designed for the production, monitoring, and harvesting of Imopoea Batatas somatic embryos. Experiences with toxicity, contamination, and mechanical damage to embryos will be discussed.

I-1001 Development of Primary Culture of Epidermal Cells from the Penaeid Shrimp, Penaeus vannamei.
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Tissue culture systems have been used in some studies on effect of hormones in growth regulation of crustaceans, but to our current knowledge no cell line have been established. In this work, we attempted to culture epidermal cells from the shrimp Penaeus vannamei which is one of the major Penaeid species used for aquaculture .
Shrimps in the premolt stage were washed with clean water and the body surface sterilized. Carapace was aseptically removed and the epidermis was carefully separated from the cuticule. The tissue was then minced into small pieces and placed in Petri dishes in a modified 199 medium, pH 7.0, supplemented with 10% fetal bovine serum, and incubated at 26°C. Osmolality was adjusted to 750 mOsm by salt addition. Proline concentration was also elevated.
Small cells (<10 µm) quickly spread out from the tissue and firmly attached to plastic. Two predominant cell types were observed : fibroblast-like cells forming non confluent networks and clumps of epithelial-like cells. Long-term maintenance primary cell cultures for more than 2 months was obtained.
The development of epidermal cell cultures could be useful for studying the role(s) of potentially active molecules in the regulation of the molt cycle and, by that way, in the growth of shrimps.

I-1002 Primary Culture of Lymphoid and Nerve Cells from *Penaeus stylirostris* and *Penaeus vannamei*.
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There is a vital need for a shrimp cell culture system to facilitate studies of viral diseases of shrimps. In our laboratory we have developed primary cultures from various organs of the *P. stylirostris* and *P. vannamei* shrimps employing several media formulations. Of the organs tested, only the lymphoid (Oka organ) and nerve cord were successfully grown in primary culture. The lymphoid cells exhibited the fastest growth rate, forming a subconfluent monolayer in five to seven days. In contrast, the nerve cells grew very slowly from the tissue explants but also showed extensive cell outgrowths after two to three weeks. Both the lymphoid and the nerve cells showed limited growth on subcultivation. The lymphoid cell cultures were successfully infected with the rhabdovirus of the penaeid shrimp. The media that best supported the growth of both cell cultures was 2X L15 supplemented with fetal bovine serum and shrimp head extract at an osmolarity of 750 to 770 mmol/kg. The two primary cultures developed in this study represent starting points for the eventual development of continuous cultures of shrimp cells. This study was supported by grants from the UH Sea Grant and College Program and the Aquaculture Development Program, State of Hawaii.

I-1003 Discovery of EGF-like factor in Mollusks.
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No continious cell lines obtained from marine invertebrates are described now. Absence of the specific stimulators of cell proliferation - growth factors - is one of the causes of failure in long-term cultivation of the marine invertebrate cells. In search of such stimulators in tissues of marine invertebrates, we succeeded in discovering in some tissues of stimulator of cell proliferation, similar to EGF, with comparatively high content of it in the tissues of Mytilus edulis. The similarity of obtained factor with EGF was shown by the substitution of [^{125}I]EGF, connected with EGF receptors on the surface of A431 cells, with analyzed extracts, as well as by the ability of this extract to induce the internalization of EGF-receptor complexes. The fraction of acid/ethanol extract of M.edulis, stimulating the cell proliferation in resting mouse fibroblasts Swiss 3T3 and DNA synthesis in primary cell cultures of mollusks, was obtained with the same conditions as EGF did when reversed-phase chromatography was used. Thus, the factor from tissues of M.edulis can be belong to family EGF-like factors. There is as yet not report of an EGF motif in mollusks, and our report is the first.

I-1004 Initiation and Promotion of Hematopoietic Neoplasia in Soft Shell Clams Exposed to Natural Sediments.
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Field studies have shown that soft shell clams (Mya arenaria) living in contaminated sediments in New Bedford Harbor, MA have a higher prevalence of hematopoietic neoplasia than clams living in less contaminated sediments. To determine the mechanism by which this phenomenon occurs, either initiation of the neoplasia or promotion of an endemic problem, a protocol modified from rat liver neoplasia studies was undertaken. Previously diagnosed clams (using an immunoperoxidase technique) were distributed into individual pots (10 clams per pot) containing sediment collected from New Bedford Harbor or a control site. The clams were distributed into 3 disease groups; clams with no evidence of the disease (stage 0 group), clams with a disease state where less than 15% of the circulating hemocytes were neoplastic (stage 1 group), and clams that were diagnosed disease-free but were then challenged with an injection of 10^3 neoplastic cells in filtered seawater (transmitted group). Disease state, mortality, and growth rates of each group were monitored over a 4 month period. The results indicate that clams which were originally diagnosed with no disease developed leukemia at about the same rate regardless of the sediment type they were living in. Those clams that were challenged with the diseased cells developed leukemia at a significantly higher rate when exposed to contaminated sediment, indicating that the contaminants in sediment from New Bedford Harbor assist in promoting the development of the disease once it has been initiated. This observation was supported by the stage 1 clams which also showed a higher rate of disease progression when exposed to New Bedford Harbor sediment. These data suggest that the significantly higher prevalences of hematopoietic neoplasia observed in New Bedford Harbor are due to the promotional properties of the contaminants (including PAHs, PCBs, and trace metals) rather than initiation of the leukemia.

I-1005 Utilization of Carbohydrates and Accumulation of Lactate in Spodoptera frugiperda Insect Cell Cultures. A. T. NAHAPETIAN T. A.Trivits, J. T. Pepe and J. R. Orton. Du Pont Merck Pharmaceutical Co.,Glenolden, PA 19036,

Present study was initiated to study utilization of glucose, fructose and sucrose and accumulation of lactate in Spodoptera frugiperda (Sf9) insect cell cultures in 162 cm2 T-flasks, 700 cm2 SteriCell vessels (O2 and CO2 permeable bags), 8 liter spinner flasks and a one liter CelliGen bioreactor during production of a recombinant protein. The cells were adapted to their respective environments for two weeks prior to the study. On day 0, stock cultures were re-suspended in fresh Grace's supplemented medium (10% fetal bovine serum, 0.1% Pluronic F68 and 4 mM L-glutamine) and they were infected (MOI=1-2) with Autographa californica nuclear polyhedrosis virus engineered to produce a recombinant protein. Non-infected cultures were used as controls for all but the bioreactor environment. Culture volumes in T-flasks, SteriCell vessels, spinner flasks and CelliGen were 50, 500, 3900 and 1300 ml, respectively. Experimental period was 7 days. Contrary to our expectations, in all four environments, under both infected and non-infected conditions, no significant declines in concentrations of glucose, fructose and sucrose were observed. Highest accumulation of lactic acid occurred in the spinner flask environment, under both infected and non-infected conditions. In the infected cultures, following a slight increase within the first 24 hours lactate concentrations in the T-flasks and SteriCell vessels remained constant during days 1-7. In contrast, in the CelliGen culture, there was a sharp decline in lactate concentration on days 0-3. On day 3, lactate concentrations in the infected spinner flask, T-flask, SteriCell and CelliGen cultures were 3.90, 2.51, 1.65 and 0.43 mM, respectively. On the same day, cell densities in the infected spinner flask, T-flask, SteriCell and CelliGen environments were, 9 x 10e5, 8 x 10e5, 7 x 10e5 and 4 x 10e6 cells/ml, respectively. It was postulated that, under the conditions of our study, probably due to highly aerobic oxidation and efficient utilization of carbohydrates by Sf9 insect cells, carbohydrates were not limited and lactate did not accumulate appreciably in all but the spinner flask environment.

I-1006 Adaptation of Orgyia leucostigma (Lepidoptera: Lymantriidae) IPRI-0L-12 Cells to Serum-free Media. S.S. SOHI, G.F. Caputo and W. Lalouette, Forest Pest Management Institute, Forestry Canada, P.O. Box 490, Sault Ste. Marie, Ontario P6A 5M7, Canada.

Insect cell cultures offer a cleaner, viable, and probably in the long run a cheaper alternative to insect larvae for producing viral insecticides. We have developed over 60 continuous cell lines from tissues of forest insects, and several of these replicate baculoviruses. But the utilization of these cell lines for the production of viral insecticides has been hindered by the high cost of media, and media supplements like fetal bovine serum (FBS). Currently, we are working on adapting our cell lines to FBS-free media. Here we report our work on the white-marked tussock moth, Orgyia leucostigma, cell line IPRI-0L-12 that replicates two baculoviruses (Sohi et al., Can. J. Microbiol. 27: 1133-1139, 1981; Intervirology 21: 50-60, 1984). These cells are grown in Grace's medium supplemented with 0.25% (w/v) tryptose broth and 10% FBS. Three other media, IPL-41, EX-CELL 400 and SF-900, and several substitutes for serum were tried. The cells have been subcultured 17 times in SF-900, and eight times in IPL-41 medium supplemented with Pluronic F-68, lipid mixture and yeast extract ultrafiltrate. Also, Serum Free Insect Medium Additive (Sigma), Bovine Embryonic Fluid (Sigma), Growth Media Supplements A, G, S, and X (GIBCO) have shown promise as substitutes for FBS.

I-1007 Viral Pesticides: Production in Serum-Free Insect Cell Culture

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For a long time, insect cell culture and baculoviruses were primarily used in research as a potential means for the production of biological control agents for insect pests. This potential was not fully explored and exploited until genetic engineering rapidly revolutionized baculovirology for foreign gene expression which signified insect cell culture as a desirable system in current biotechnology.

To date, the production of viral pesticides for commercial applications employs larvae as a host for *in vivo* processes. This traditional method of production presents many known disadvantages. The use of insect cell culture as a host for entomopathogenic viruses offers many advantages: consistent process control, product cleanliness and cost effectiveness of downstream processing, including recovery and formulation of the final product. However, to reduce the cost of virus production in cell culture for viral pesticides, it is of paramount importance to develop low cost serum-free medium (SFM) that will support virus production yields having the maximum number of polyhedra inclusion bodies (PIB) and biological activity equal to PIB produced *in vivo*.

A low cost prototype, BioPesticide SFM, for the growth of a variety of insect cells (Sf9, Sf21, *Tn*-368, *L. dispar* [Ld652Y] and *H. zea* [Hz1B3]) and production of baculoviruses (*Ac*MNPV and *Op*MNPV) was developed and evaluated in large scale, commercially available Celligen™ (New Brunswick) and Chemap airlift cell culture bioreactors. When first instar *Trichoplusia ni* (*T. ni*, a cabbage looper) larvae were challenged with PIB produced in bioreactors using prototype BioPesticide SFM, the LC_{50} of PIB produced in cell culture was comparable to PIB produced *in vivo*. The titers of extracellular virus (ECV) progeny produced in insect cell culture using prototype BioPesticide SFM were equal to the ECV produced *in vivo*. These studies suggest the *in vitro* production of viral pesticides may be a cost-effective alternative to *in vivo* production.

I-1008 Ecdysteroid-Stimulated Differentiation in a Lepidopteran Cell Line Derived from Imaginal Discs. P. PORCHERON[1] and H. Oberlander[2]. [1]Laboratoire de Biochimie, Ecole Normale Supérieure, 75005 Paris, France. [2]Insect Attractants Laboratory, USDA, Gainesville, FL 32604.

The availability of insect cell lines of defined origin provides new potential for studies concerning endocrine control of development of insects at the cellular and molecular levels. Lynn and Oberlander successfully established some years ago a cell line from imaginal discs of Plodia interpunctella (IAL-PID2) which responded to the molting hormone, 20-hydroxyecdysone (20E). The hormone inhibited in a dose-dependent manner the proliferation of IAL-PID2 cells and induced morphological differentiation leading to epithelial-like arrangement of the cells. In parallel, the 20E selectively stimulated the uptake by the cells of N-acetyl-D-glucosamine (GlcNAc), and the rapid incorporation of this amino-sugar into a 5,000 dalton GlcNAc rich glycopeptide secreted by the cells. Accumulation of this peptide was prevented by the use of teflubenzuron, a potent chitin synthesis inhibitor. A similar glycopeptide was also observed in intact imaginal discs treated with 20E in vitro, under conditions that induce chitin synthesis. Kinetic studies indicated that this molecule behaved as a precursor of high molecular weight glycoproteins. Therefore, the hormone-dependent glycopeptide could represent the basic unit of extracellular matrix proteoglycans, called chitinoproteins, involved in vivo in cuticle synthesis by differentiated epidermal cells. This cell culture model is promising for in depth study of some cellular and molecular aspects of ecdysteroid-regulated processes of differentiation of insect epidermal cells.

I-1009 In Vitro Culturing of the Verson's Gland of *Manduca sexta*, and the role of 20-hydroxyecdysone in Cell Growth and Pupal Commitment. K. L. HORWATH, Department of Biological Sciences, State University of New York at Binghamton, Binghamton, NY 13902

Specialized epidermal cells (Verson's glands) from the tobacco hornworm, *Manduca sexta*, are responsible for the production of the proteinaceouse cement layer which coats the epicuticle at ecdysis. Following larval to pupal transition the composition of this secretion differs from that during a larval molt. Fluorograms from pulse labeling of the secretory cells of penultimate, 4th and final 5th larval instars indicate that protein synthesis is coincident with the molting surge of ecdysteroids. In vitro culturing of the glands from newly ecdysed 4ths, and day 2 5th instars with 20-hydroxyecdysone, 20HE, (2.5 µg/ml 20HE in the presence of 1 µg/ml juvenile hormone, JH) confirms ecdysteroid induced protein synthesis, with no such response with JH alone. Moreover, in vivo tranplantation studies show that secretory cells undergoing larval-pupal transition (day 1 5ths) synthesized both larval and pupal products simultaneously during an ecdysteroid enforced molt. In vitro culturing of glands from day 1 5th instars with 20HE and JH resulted in concurrent synthesis of both stage specific products. Preliminary results of similar culturing of such cells in the absence of JH supports 20HE involvement in pupal commitment.

I-1010 Correlation of Fat Body Histochemistry and Cell Morphology in Primary Cultures to Developmental Stages of *Manduca sexta* and *Tenebrio molitor*. C. M. EASTON and K. L. Horwath, Department of Biological Sciences, State University of New York at Binghamton, Binghamton, NY 13902

Histochemical assessment of fat body tissue from the moth, *Manduca sexta*, reveals that the larval-pupal transition is accompanied by a reduction of stored glycogen, increases in the levels of stored lipids and proteins, and the formation of protein granules. In contrast, the beetle, *Tenebrio molitor*, exhibits extensive protein storage in granules throughout larval development, well prior to the larval-pupal transition. Primary cultures of fat body established from both species display heterogeneous cell types with the presence of protein granules correlating with morphologically distinct "globular" cells. These rounded, large (40-100 µ), heavily vacuolated cells, also stain strongly for lipids and glycogen, closely resembling mature trophocytes. Other cell types observed in primary cultures of fat body stain only moderately for lipid, glycogen, and protein, and include spindle shaped cells (20-70 µ), and large stellate cells (60-180 µ). The distinct morphologies observed in primary cultures may indicate either truly distinct cell types or reflect differences in the degree of cellular differentiation. Furthermore, our in vivo and in vitro characterizations suggest that the coleopteran fat body, unlike that of lepidoptera, is suited for extensive protein storage throughout larval development

I-1011 Survival Response of TN-368 Lepidopteran Cells to Psoralens and UVA Light. T.M. KOVAL and D.L. Suppes. Division of Radiation Oncology, Mayo Clinic and Mayo Foundation, Rochester, MN 55905

TN-368 lepidopteran insect cells exhibit a pronounced resistance to several physical agents such as x and gamma rays, 254 nm ultraviolet light, and heat, as well as a variety of chemical agents. Studies with the chemical agent mitomycin C implied that TN-368 cells may have some deficiency in recovering from damage caused by DNA-DNA and/or DNA-protein crosslinking agents. Although the TN-368 cells were much more resistant to mitomycin C than mammalian cells, their ability to undergo cellular recovery during a period of liquid holding was absent. This was in contrast to results with virtually all other chemical and physical agents tested. To further examine the role of crosslinks, investigations were initiated using a linear psoralen, 8-methoxypsoralen (8MOP), and an angular psoralen, angelicin, in combination with 365 nm ultraviolet (UVA) light. It is well documented that under such conditions both psoralens covalently bind to DNA to form monoadducts, but only 8MOP is capable of forming diadducts to crosslink the DNA. The lethal effects of these treatments were studied in parallel in the TN-368 insect and V79 Chinese hamster cells using a colony formation assay. TN-368 cells were much more resistant than V79 cells to combinations of either 8MOP or angelicin with UVA light. Both cell lines underwent considerably more UVA-induced killing following 8MOP rather than angelicin treatment, presumably due to crosslinking damage. The survival response of the TN-368 cells was further investigated under liquid holding conditions. Following short periods of holding, no recovery was observed for either psoralen plus UVA treatment. While this would suggest that DNA crosslinking is not the explanation for the lack of recovery, longer liquid holding periods will be examined before this conclusion is reached. (Supported in part by USPHS grant CA 34158 from NCI, DHHS).

I-1012 Stable Transfection of Mosquito Cells with a DNA Construct containing the Dihydrofolate Reductase Gene. A.M. FALLON, F.A. Shotkoski and Y.-J. Park. Department of Entomology, University of Minnesota, St. Paul, MN 55108.

Methotrexate-resistant Aedes albopictus mosquito cells, in which the dihydrofolate reductase (DHFR) gene was amplified to approximately 1200 copies per cell, were used as a source of DNA for cloning the DHFR gene. The mosquito DHFR coding sequences spanned 614 bp, and contained a single 56 bp intron at a conserved position near the 5'-end of the gene. In contrast, vertebrate DHFR genes contain 5 conserved introns and span over 30 kb. The DNA sequence upstream of the insect DHFR gene was AT-rich, contained 4 T-rich motifs, and lacked the GC-repeats typical of vertebrate DHFR regulatory regions. Three predominant transcription initiation sites were identified using primer extension and RNAase mapping techniques. A number of minor initiation sites were also present. The DHFR gene and its regulatory sequences was subcloned into an hsp-CAT plasmid to generate pDHFR/hsp-CAT. After transfection of wild type mosquito cells with this recombinant plasmid, methotrexate selection was applied to select transformed clones. The presence of pDHFR/hsp-CAT was ascertained by induction of CAT (chloramphenicol acetyltransferase) activity by activation of the Drosophila heat shock protein (hsp) promoter at 41°C. Populations of cells derived from these clones retained transfected DNA in stable form for over 16 weeks.

I-1013 Multiplication of a Baculovirus of Malacosoma disstria (Lepidoptera: Lasiocampidae) in a Homologous Cell Line.

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Outbreaks of the forest tent caterpillar, Malacosoma disstria, appear at irregular intervals throughout much of the United States and Canada. Heavy infestations cause considerable defoliation and loss of growth in host trees, and the migrating larvae are a great nuisance around houses, and in parks and campgrounds in infested areas. Also, defoliation by M. disstria reduces the yield of non-fiber products, such as maple syrup and syrup products. We have established a continuous cell line, IPRI-MD-66, from the hemocytes of this insect (S.S. Sohi, Can. J. Zool. 49: 1355-1358, 1971) and have reported the multiplication of a microsporidium in it (S.S. Sohi and G.G. Wilson, Can. J. Zool. 54: 336-342, 1976). Here, we report the replication of M. disstria nuclear polyhedrosis virus in this cell line. The virus inoculum was prepared by homogenizing M. disstria larvae infected with the virus. Cells in log phase were centrifuged at 135 xg for 5 min and the cell pellet was resuspended in 1 ml of virus inoculum. The cells were kept at 28°C before and after inoculation with virus. Occlusion bodies appeared in the nuclei of cells one day post inoculation, and cytopathological changes were typical of NPV infection. The virus has been serially passaged eight times and the level of infection fluctuated from 40 to 67%.

I-1014 Cell Lines Used in Baculovirus Expression Vector Selection.

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Baculoviruses are capable of replication and plaque formation in a number of cell lines. The development of baculoviruses as expression vectors for foreign gene expression renewed the interest in the study of insect cell lines. Because occlusion negative plaques are difficult to visualize, expression vectors have been developed containing the ß-galactosidase gene that when expressed causes the substrate around plaques to turn blue. Autographa californica nuclear polyhedrosis virus (AcMNPV-E2), and two recombinants with the ß-galactosidase genes were the inocula. The plaque assays were performed using Trichoplusia ni (TN-368), and Spodoptera (SF9 and IPLB-SF-21AE) cell lines to compare time of appearance, size and number of plaques formed. Another occluded negative virus was also studied for appearance time in the different cells. TN-368 cells produced occluded, non-occluded and blue recombinant plaques several days sooner than either Spodoptera line. The appearance of new plaques was also different for the three cell lines. The non-occluded, non-blue recombinant was easier to identify in TN-368 cells compared to Spodoptera cells, but for only a short time period before the cell monolayer deteriorated. The occ^+ and occ^- blue plaques were twice as big in TN-368 as in the Spodoptera cells.

I-1015 **Large Scale Process Optimization for the Production of Recombinant Proteins in the Baculovirus Expression Vector System**

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By optimizing culture conditions, infection parameters, and use of superior second generation serum-free medium (SFM), such as Sf-900 II SFM, we have achieved enhanced yields of recombinant proteins in large scale bioreactors utilizing the baculovirus expression vector system (BEVS). Control of pH and dissolved oxygen, and maintenance of essential amino acids and carbohydrates at non-rate limiting concentrations are demonstrated in commercially available bioreactors, such as the New Brunswick Celligen™ stirred tank (2 to 5L) and Chemap Airlift (5 to 20L) when combined with SFM formulations which have been developed for use in high density cell culture. With recombinant virus expressing ß-Galactosidase (clone VL-941 rß-GAL) and EPO (clone BV-EPO), we have achieved 2 to 10 fold increases in total product yield when compared with serum supplemented and first generation serum-free controls. The expression of α-Galactosidase (clone AcCC1) and rotavirus VP-6 (clone BRV6-1) proteins has also been improved resulting in comparable or increased yields. Secreted or non-secreted proteins expressed in Sf-900 II SFM exhibited no significant differences in structure or bioactivity when compared to product produced under serum supplemented conditions. Elimination of serum reduces medium cost, simplifies downstream processing and purification of the final product. These are key considerations when determining the cost effectiveness of a process or in qualifying a recombinant product for use as a potential therapeutic agent.

I-1016 **New Developments in BEVS Accessory Package Applications**

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Recent advances in the serum-free culture of insect cells and baculovirus-based biopesticides and expression of foreign genes have improved cell yields, recombinant product quality and production, and purification efficiency. An accessory package of products for the cultivation of insect cells, co-transfection of transfer vectors, purification of recombinant virus and viral DNA, and recombinant protein production has provided additional benefits to both research and industrial specialists. This paper describes further applications of these products in a variety of cell lines, vectors and bioreactor configurations.

A manual introducing basic BEVS materials and techniques has been produced to assist those interested in applying BEVS technology to their protein expression needs. Novel applications of the plaquing reagents, employing methylumbelliferill and formazan based selection protocols have been examined. Improved techniques in the co-transfection of insect cells resulting in recombinant baculovirus employing a variety of mediators of DNA uptake have been developed. Rapid, convenient techniques of baculovirus genome purification and examination should allow "real-time" analysis of the DNA from the progeny virus of infected cultures. Both the quantitation of viral DNA (allowing estimation of virus titer) and qualitative analyses of restrictively digested genomes on mini-agarose gels may be produced on a same-day basis. This capability permits monitoring infection kinetics in research, development and production, and is especially useful in large scale bioreactor and biopesticide applications.

I-1017 A Simplified Serum-Free Powdered Insect Medium with Improved Performance Characteristics and Multiple Cell Line Applicability. V. CALDWELL, L. Tabor, D. Kern. HyClone Laboratories, Inc. 1725 S. HyClone Rd. Logan, Utah 84321

With the increasing popularity and use of the baculovirus recombinant protein expression systems there has been increased interest in serum-free media formulations for insect cell culture support. Our data reveal that medium produced from the powder form of CCM3™ serum-free insect cell culture medium often yields adaptation times with Sf9 cells (ATCC CRL 1711) comparable to supplemented Grace's medium with 10% FBS. Improved adaptation performance has been observed as compared to the commercially available serum-free media evaluated. Maximum cell densities in culture with CCM3™ have also proven comparable to the performance observed with the traditional FBS containing medium. Maximum cell densities attained in culture proved greater with CCM3™ than with the commercially available serum-free media evaluated. Data also indicates that medium produced from the powder form of CCM3™ yields cell culture growth comparable to the supplemented Grace's medium with 10% FBS for the original TN-368 cell line as well as the commercially available BTI-TN-5B1-4 cell line.

I-1018 Insect Cell And Baculovirus Production in Serum-Free Media. B.W. BELISLE, C. Celeri, K. Tang[1], T. Montgomery, and T. Gong[2]. [1]American Cyanamid Company, San Leandro, CA 94577 and [2] JRH Biosciences, Woodland, CA 95695

The baculovirus expression vector system (BEVS) is recognized as a viable method of obtaining high level production of biologically active recombinant proteins and for production of virus for use as biological control agents. Sf9 cells (*Spodoptera frugiperda*) have been the host cells of choice, but other cell lines are being investigated as possible alternatives for BEVS applications. Cell lines from four Lepidopteran species adapted to growth in commercially available media (EX-CELL 400 or 401; JRH Biosciences) were examined for serum-free cell growth and virus production. Cells were routinely carried as serum-free seed stocks, in some cases for >100 passages. Cultures were maintained in a variety of vessels, including T-flasks, shake flasks, sparged spinner flasks, and airlift bioreactors at scales ranging from 5 ml to 40 liters. Cells from *Spodoptera frugiperda* (Sf9), *Lymantria dispar* (IPLB-LdFB), *Trichoplusia ni* (TN-368 and High Five) and *Bombyx mori* (BmN) were infected with either AcMNPV (*Autographa californica* nuclear polyhedrosis virus) or LdMNPV (*Lymantria dispar* nuclear polyhedrosis virus) under serum free conditions, and the extracellular and occluded virus production monitored. These cell lines (and most other insect lines we have studied) have adapted easily to growth in the serum-free media used with only minor modifications occassionally required. Cell growth and virus production are comparable or better than those observed in serum based media.

I-1019 Establishment of A Midgut Cell Culture from Lepidoptera. S. SADRUDDIN *, R.S. Hakim * and M. Loeb **. *Dept.of Anatomy, Howard University, 520 W. St. N.W. Washington D.C. 20059. **Insect Reproduction and Hormone Lab., USDA, BARC E., Beltsville, MD. 20705

We have established a culture system for midgut epithelial cells. Sections of midgut were taken from Manduca sexta third instar larvae and cultured in modified Grace's medium containing 7% fetal bovine serum, 20 hydroxyecdysone (20 HE) and fatbody tissue. During the first week, the epithelium becomes opaque and detaches from the underlying framework of muscle, trachea and nerve. By 10 days goblet, columnar and stem cells appear in the media. Columnar and goblet cells undergo periodic rounding and acquire opaque granules. This is the time when the greatest number of stem cells are present. Mats of differentiated epithelial sheets form without the underlying matrix. These show the characteristic pattern seen in vivo in which individual goblet cells are surrounded by a single cell recticulum of columnar digestive cells. Our cultures have been maintained for as long as 10 weeks. Without 20HE and fatbody, midgut epithelial cells survive to detach from the matrix but grow very slowly if at all. This culture system offers a model for studying midgut pattern formation and epidermal stem cell growth and differentiation.

I-1020 Development of a Transposition Assay for Lepidopteran Transposons Using a Baculovirus Genome as a Target. M.J. FRASER, K. Boonvisudhi, K. Pecen, P. Brzezinski, T. Ciszczon, J. Pancheri, L. Cary, and H. Wang. Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556.

Several unique features of Baculovirus replication and assembly permit transposon mediated mutagenesis of the virus genome generating viable virus. A recurring spontaneous mutant phenotype called FP can be generated by host transposon insertions within a gene encoding a 25 Kda protein of as yet undefined function. In past analyses we have characterized two transposons inserting within the 25K gene and noted a target site specificity for the insertion event that is common to both types of transposon. PCR analyses of genomic copies of one transposon confirmed that the target site specificity for the insertion event was a quality of the transposon and was not influenced by the virus. This lab is currently adapting the Baculovirus/host system for molecular analyses of the insertion and excision events of these Lepidopteran transposons. In the present report we demonstrate that transposition of plasmid-carried, lacZ-tagged copies of Lepidopteran transposons can be followed into the Baculovirus genome. The insertion event retains the characteristic target site specificity associated with these transposons. This study provides a basis for the continued development of the Baculovirus/host system for analysis of target site-specific insertion of these eukaryotic transposons.

P-1001 Splicing Abnormality of a Chloroplast Gene Transcript in Albino Pollen Plants in Rice.
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National Institute of Agrobiological Resources, Tsukuba, Ibaraki 305, JAPAN

Albino plant regeneration is one of the major abnormality in monocot crop tissue culture (rice, wheat, barley), especially by anther culture. The reason of the albino regeneration is attributed to be deletions of chloroplast DNA occured in tissue culture process. However, the albino regeneration occures up to 100% among regenerated pollen plants in rice. Therefore, other mechanism of the phenomenon should be investigated. We have studied the expression of chloroplast genes in rice calluses and regenerated plants by Northern hybrydization. All most all the genes were depressed in the transcriptional level in callus and early regenarated process. However, some new transcripts were observed among albino calluses and plants. A large accumulation of 2.7kb transcript was detected using the tobacco chloroplast DNA clone (pTB28-1; harboring *trn*1-*rpl*23-*rpl*2-*trn*H*-*psb*A*) as a probe. Detail analyses of the 2.7kb transcript revieled that the splicing of the intron of *rpl*2 gene dose not occur in albino. The 2.7 kb transcript is detected in 93% of examined 136 albino pollen plants derived from 22 different hybrids and varieties and 33% of seed callus derived albino offsprings, which have been determined as single recessive nuclear gene mutations. These results also indicated the existence of the hot spots of the mutation induced by the tissue culture and the one of them is related to the splicing enzyme coded in the nuclear genome.

P-1002 Genetic Engineering of Osmolyte Biosynthesis Genes in Tobacco.
R.K. JAIN*, S. Jana* and G. Selvaraj**. *Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon, Canada S7N OWO.** Plant Biotechnology Institute, Saskatoon, Canada S7N OW9.

Glycine betaine (betaine), a non-toxic quaternary ammonium compound, is accumulated in many procaryotes and eucaryotes in response to osmotic stress. Recent studies with bacterial systems have confirmed the utility of betaine in alleviating growth inhibition caused by osmotic stress. In some salt- and drought-tolerant plants and in bacteria betaine is synthesised from choline via betaine aldehyde. However, this pathway is either absent or incomplete in most crop plants. Genetic manipulation of crops to synthesise betaine may lead to improved osmotolerance. The benefit to mankind, if this approach succeeds, is evident as drought and salinity limit the crop productivity to a very significant extent. Betaine aldehyde dehydrogenase (BADH) catalyses the final step in betaine biosynthesis. A bacterial gene encoding for BADH (*betB*) and a cDNA for BADH from a halophytic plant were transferred to tobacco through *Agrobacterium*-mediated transformation. Transgenic plants were identified by their kanamycin resistant phenotype, neomycin phosphotransferase assays and also by amplifying the bacterial *betB* sequence by polymerase chain reaction. BADH activity measurements in transgenic plants carrying the bacterial *betB* gene indicated a lack of expression and immunoblot analysis did not identify a BADH-like protein. This unexpected observation, particularly in the face of a high degree of structural similarity between bacterial and plant BADHs, raises questions regarding gene expression, regulation and enzyme assembly of *betB* gene in a heterologous milieu. The results obtained are informative in the general context of transgenic gene expression.

P-1003 Characterizations of Rice Cell Suspension Cultures of Mutants for Lysine and Protein Levels.
G. W. Schaeffer, USDA, ARS, Beltsville, Md 20705

Cell suspension cultures of rice mutants recovered from anther calli insensitive to inhibitory levels of lysine plus threonine (L+T) and S(2-aminoethyl) cysteine were developed to study lysine metabolism and protein systhesis and processing. One unique characteristic of the mutant is a higher level of protein exported into the culture medium. The mechanism for the increased protein export is not yet clear. The amino acid composition of electrophoretically separated proteins demonstrate some unique proteins in the mutant including some with enhanced lysine. Specific proteins exported into the medium include the β1-3 glucanases and chitinases. Enzymes have been isolated from leaves and the culture medium. Enzymes were purified to near homogeneity and polyclonal mouse antibodies produced. Western blotts demonstrate the devleopmental expression of these hydrolases. The mutant cell culture line exports about 4 times as much enzyme as the corresponding control at 7 and 10 days after inoculation. The conclusion is that the mutant line is valuable for the study of transcription, protein processing and the control of gene expression.

P-1004 Accumulation of Flavonoids is Essential for UV-Resistance in *Arabidopsis*.
R. LOIS and B. Buchanan. Dept. Biological Science, California State University, Fullerton, CA 92634 and Plant Biology Dept., University of California, Berkeley, CA 94720

The recent depletion of the atmospheric ozone layer has awakened an interest in the biological effects and responses to UV-B radiation. Little is known, however, about the mechanisms that protect plants from the damaging effects of UV-B. We have used *Arabidopsis* as a model system in an effort to elucidate the molecular mechanisms of plant defenses against UV-light damage. Several *Arabidopsis* ecotypes were found to respond to UV-radiation by increasing the levels of accumulated flavonoids which absorb in the UV-region. The levels of flavonoid accumulation in different tissues correlate well with the degree of UV-resistance of each tissue . A mutant was isolated which displays a marked increase in sensitivity to UV-B radiation but shows no changes in sensitivity to other stresses such as heat, cold, root hypoxia, osmotic shock or pathogen attack. Genetic analysis demonstrated that this mutation segregates as a single Mendelian locus, indicating that a single gene has been affected. This mutant was found to lack a small group of flavonoids (flavones and flavonols) that correspond to those compounds that accumulate in response to UV-radiation in wild type *Arabidopsis* plants. These findings indicate that one or more of these compounds must play an essential role in the plant's resistance to UV-B radiation.

P-1005 Differential Response of Resistant and Susceptible Wheat Calli Cultures to Russian Wheat Aphid Phytotoxin. M.M.RAFI and R.S.Zemetra. Dept. of PSES, Univ of Idaho, Moscow, ID 83843.

Growth patterns and one dimensional protein profiles of Russian wheat aphid (RWA) phytotoxin resistant (PI 137739) and susceptible ("Stephens") wheat calli cultures derived from immature embryos were investigated. RWA phytotoxin was extracted using Tris/NaCl solution and fractionated in a Sephadex G-25 column. Calli were maintained on a solidified MS medium supplemented with 10 µM 2,4-D, 3% sucrose, 500 mg casein hydrolysate and 100 mg glutamine and placed in the dark. Calli cultures were exposed to 50, 100 and 150 µL of fraction three of the RWA toxin for a period of six weeks and changes in growth and protein synthesis were determined. Application of 50 µL of the toxin revealed differential growth patterns between resistant and susceptible calli cultures. Application of 150 µL of toxin resulted in total arrest of calli growth, indicating an extreme level of toxicity. Growth of PI 137739 callus was consistently higher than "Stephens". There was an increase of 25% fresh callus growth of PI137739 over "Stephens" at week six. Protein profiles of toxin treated resistant and susceptible calli were distinctly different as revealed by the Phast SDS-PAGE system. The differences between the protein profiles of resistant and susceptible calli indicates a potential protein(s) response to the RWA phytotoxin.

P-1006 Somatic Hybrids and Cybrids between *Senecio fuchsii* Gmel. and *Senecio jacobaea* L. G.R. WANG and H. BINDING, Botanical Institute, Christian-Albrechts-University, Kiel, W-2300, F.R.G.

Somatic hybridization between *Sencio fuchsii* and *S. jacobaea* was investigated. Regeneration from protoplasts had been achieved with both species (Binding *et al.*, 1981 and 1991). In order to establish visual markers, albino mutants of *S. jacobaea* (one plastid and one nucleus mutant) were selected after treatment of protoplasts with nitromethylurea. Fusion was obtained *via* the agarose sandwich lense technique with high Ca^{++} and high pH (Binding *et al.*, 1988). Several regenerant lines were selected by pigmentation and morphological characteristics. The constitutions of five hybrid lines and two cybrid lines were confirmed by chromosome numbers, patterns of isozymes (peroxdase, esterase, malate dehydrogenase, glutamic-oxalacetic transaminase), and RFLPs. Restriction fragment patterns of total NDA were explored by specific probes of nuclear DNA and chloroplast DNA. Plastids had segregated in the shoots investigated. The two cybrids each possessed 2n=2x=40 chromosomrs. One of them contained the *S. jacobaea* nuclear genome and *S. fuchsii* plastome, the other one contained *S. fuchsii* nuclear genome and *S. jacobaea* plastome. Analyses of RFLPs with mtDNA probes are in progress. The development of plants in the greenhouse is being followed.

P-1007 Molecular and Quantitative Studies on Foreign Gene Expression. R.H. SMITH and E.C. Ulian. Dept. of Soil & Crop Sciences, Texas A&M Univ., College, TX 77843.

Twenty-nine primary transformed petunia plants were examined for phenotypic expression of NPTII and GUS genes. Molecular studies established copy number and presence of the foreign genes. Quantitative studies on enzyme expression showed varying levels of gene expression for the 2 genes and indicated copy number did not always influence the level of gene expression. Studies on 7 mature plants over a 4 month interval generally resulted in consistent gene expression; however, 2 plants showed varying levels of gene expression. Studies on methylation indicated that methylation events were responsible for the absence of gene expression in most, but not all, plants.

P-1008 Expression of the Sulfur-Rich Brazil Nut 2S Albumin in Transgenic *Vicia narbonensis* Plants. T. PICKARDT, I. SAALBACH, F. MACHEMEHL, G. SAALBACH, O. SCHIEDER and K. MÜNTZ. Institute of Genetics and Crop Plant Research/Gatersleben and Institute of Applied Genetics, Free University/Berlin, Germany.

Breeding as well as genetic engineering programs aim at the improvement of the amino acid composition of legume seed proteins. The gene for the 2S albumin from Brazil nut (*Bertholletia excelsa*) seems highly suitable for use in a gene transformation approach because the protein contains 26% sulfur-containing amino acids. We have synthesized the protein coding region of that gene in vitro according to a cDNA sequence (Altenbach et al. 1987) and put it under the control of the CaMV 35S promoter as well as two seed specific promoters of field bean seed proteins. The chimeric 2S albumin genes were inserted into binary vectors and transformation experiments were carried out with the supervirulent *Agrobacterium tumefaciens*-strain EHA101 (Hood et al. 1986). Tissue culture and regeneration of transformed fertile plants of *Vicia narbonensis* were based on the protocols of Pickardt et al. (1991). The transformation of the 2S albumin gene was verified by Southern-blots, the protein was detected using immuno-blotting. So far three transgenic *V.narbonensis* plants containing the 2S gene controlled by the CaMV35S promoter could be grown to maturity. The 2S albumin expression was observed in the selfed progeny of two of them. In these plants the foreign seed protein was detected in leaves (approx. 0.3% of total protein), seed coat (0.1%) and cotyledons (0.01%).

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P-1009 Somatic embryogenesis and complete plantlet regeneration in oil palm from inflorescence explants. N. GUZMAN and S. Umaña. Agricultural Services and Development (ASD). Palma Tica. Apartado 30-1000. San José, Costa Rica, América Central.

Somatic embryogenesis from inflorescence explants has been documented in two palm species: Cocos nucifera and Euterpe edulis. Experiments were conducted to investigate the possibility of using inflorescence culture for the in vitro propagation of elite palms selected by the oil palm breeding program of Palma Tica. Segments (0.1-0.25 cm) of rachillae from young female and male inflorescences were used as explants. The stage of development of the inflorescences was found to be critical in obtaining an earlier and efficient callus response. Two types of callus were obtained. A nodular callus developed after explant swelling and separation of the floral buds of the young spikelets. This callus multiplied by the formation of a large number of small nodules that in appropiate media gave rise to embryogenic structures, very similar to those obtained with leaf explants in other laboratories. Embryogenic callus could be multiplied and complete plantlets with well defined root and shoot poles were obtained from polyembryogenic structures. When very young explants were used a compact, white calloid (called iPE) was obtained directly 8 weeks after planting. This calloid gave rise to proembryos which differentiate to obtain complete plantlets. Plantlets have been regenerated from a number of elite genotypes of oil palm using inflorescence explants.

P-1010 Coffee Somatic Embryogenesis in Liquid Cultures. M.R. SÖNDAHL and C. Noriega, DNA Plant Technology Corp, 2611 Branch Pike, Cinnaminson, NJ 08077.

Despite coffee's great commercial importance, a commercial micropropagation method is not available for this crop. DNAP (USA) and SPIC (India) have embarked on a program to develop a process for large-scale micropropagation of coffee.

Friable embryogenic tissue (FET) isolated from coffee leaf explants and maintained on solid medium for 3 months was inoculated in liquid medium for proliferation and embryo differentiation. Cell mass increase was accomplished in a medium containing 0.25 mg/l 2,4-D and 0.1 mg/l KIN. Liquid medium was replaced after the first 4 weeks in culture and globular embryos were observed following additional 4 weeks. At this time the liquid medium was changed for the same basic medium supplemented with 2.6 mg/l ABA and replaced with fresh medium 4 weeks later.

FET cultures initiated at a low density increased 20 fold the packed cell volume in 12 weeks. After this period, torpedo stage embryos were observed at a low frequency. Culture kept without any medium exchange for additional 9 weeks differentiated entirely into embryos. Somatic embryos at different stages of differentiation were subcultured into liquid maturation medium for 4 weeks with a two-week subculture schedule. Mature embryos were plated on solid germination medium as individual embryos or in clusters. Eight weeks later, individual embryos increased 2-3 fold in size and presented normal morphology. In contrast, embryos plated in clusters were characterized by a delayed growth. Preliminary data revealed a yield of 2,500 embryos from an original inoculum of 0.2 g f.w. FET cells. Extrapolating this initial result, to achieve a micropropagation target of 1 million coffee embryos would require the use of 80 g f.w. FET cells cultured in four 5 l bioreactor vessels.

P-1011 Observation of Cultured Tissues of Plant by the Cell Processor with Laser. Y.Tahama Laboratory of Plant Pathology, School of Bioresorces, Hiroshima Prefectural University, Shobara 727, Japan.

The cell processor with a laser is a new type of microscope produced by Hitachi. Cultured cell suspension of carrot callus and cultured protoplast of tobacco were used. A drop of the carrot callus on the cover glass was observed with the cell processor on November 22, 1990. The cells consisting of nucleus, protoplasm and cell wall were observed at the first stage of experiment. The nucleus was an aggregation of small-round bodies; and the cell wall string-like bodies. When the laser beam was focused on nucleus, it dispersed. The dispersed particles moved and they showed the tendency of aggregation again. The specimen was stored in 5 °C for 4 days and was observed by the processor. The cell was greately changed, the cell wall and protoplasm were divided into small round particles. When one drop of distilled water was added onto the dried glass, the cell transformed to round bodies and they moved vigorously, just like mycoplasma. A drop of protoplast of tobacco was observed on November 27. Round ball-like protoplast was observed. When a protoplast was irradiated with a laser beam at 10:27, it broke into round bodies. Passing through the time, the round bodies became smaller at 11:25 and they moved. When the specimen was dried on the glass at 11:55, hypha-like bodies were observed. When one drop of water was added onto the specimen at 12:05, the hypha-like bodies suddenly disappeared and returned back to the former round bodies and moved.

P-1012 Micropropagation of *Citrullus lanatus* in a Membrane-based, Continual-Flow Liquid Bioreactor. J.W. ADELBERG[1], B.B Rhodes[1], S.A. Hale[2] and R.E. Young[2], Horticulture[1] and Agricultural and Biological Engineering[2] Departments, Clemson University, Clemson, SC 29634.

A membrane-based bioreactor system for plant micropropagation has been developed. This system allows for the use of filter sterilized liquid medium, sampling of medium and gaseous phase, and modification of either phase during culture cycle without disturbing plant tissue or asepsis. Shoot cultures of watermelon (*Citrullus lanatus*) show increased fresh weight and dry matter accumulation in response to weekly media changes after 28 days of growth in the bioreactor. In these tissues, percentage dry matter was lower than agar controls and vitrification was apparent. Removing benzyladenine from media formulation and providing ventilation allowed reduced vitrification of liquid/membrane cultured materials. Percentage dry matter of bioreactor cultured materials was equal to or greater than agar when daily replacement of gaseous phase was performed. A necrosis/chlorosis syndrome affects larger leaves in both agar and liquid cultured material. Calcium and manganese are in excessive concentrations in necrotic tissues. Liquid cultured materials also contain excessive magnesium, potassium, and iron levels, where agar cultured materials are not affected in this manner.

P-1013 Micropropagation of Flowering Dogwood (Cornus florida L.) : Effect of Mineral Source and Hormone Level. V. DECLERCK and S.S. Korban. Department of Horticulture, University of Illinois, Urbana IL 61801.

Cultures of Flowering Dogwood (Cornus florida L.) were established in vitro. Nodal segments were collected from greenhouse-grown plants. Explants were cultured on a basal medium containing Staba vitamins, 20g/l sucrose and 6.5g/l agar at pH 5.6. The following treatments were used: - a) 7 different sources of macroelements; explants were cultured on media containing Murashige and Skoog full [MS] and half concentration [1/2 MS], Chu [N6], Lepoivre [LP], Anderson [AND], Nitsch and Nitsch [NIT] and LLoyd and McCown [WPM]). All media were supplemented with 0.5 mg/l benzylaminopurine (BA) and 0.1 mg/l indolebutyric acid (IBA). - b) explants were subjected to 8 different hormone combinations of BA (at 0.25, 0.5, 0.75 and 1 mg/l) and with or without IBA (ratio of BA:IBA = 5). All media were supplemented with macroelements of WPM.
After three subcultures on the same medium, proliferation data were recorded. It was determined that the optimum proliferation response was observed on media containing macroelements of WPM. The concentration of BA at 0.75 mg/l without auxin was found the best. The proliferation rate recorded after 6 weeks of culture averaged 3.4 shoots/explant for the second subculture and 4.2 shoots/explant for the third subculture.

P-1014 In Vitro Nutritional Studies to Develop an Optimum Growing Medium for Alstroemeria. M.P. BRIDGEN, G.C. Elliott and M.A. Smith. Department of Plant Science, U-67, University of Connecticut, Storrs, CT 06269. U.S.A.

The in vitro growth and development of Alstroemeria were evaluated on a modified Murashige and Skoog medium containing varying amounts of calcium, iron, magnesium, phosphate, or nitrogen. Calcium was added as calcium chloride at levels from 0 to 3 mM; optimum growth occurred between 0 and 0.75 mM. Iron was added as ferric EDTA at levels from 0 to 1 mM; concentrations from 0.01 to 0.5 mM produced maximum growth. Magnesium, added as magnesium sulfate, ranged from 0 to 15 mM. The highest concentration of magnesium produced the greatest fresh weight. Phosphate was added as KH_2PO_4 from 0 to 2.5 mM. Explants supplied 1.25 or 2.5 mM P produced the most shoots and growing points and had greatest fresh weights. Nitrogen was added as nitrate and ammonium from 20 to 80 mM at different nitrate:ammonium ratios. There was no response to different nitrate:ammonium ratios; however, a positive linear response to rate was found within the range studied. After considering these results and comparing ratios between elements a recommendation can be made for Alstroemeria: 20 mM ammonium nitrate, 1.25 mM potassium phosphate, 19 mM potassium nitrate, 20 mM ammonium chloride, 0.3 mM calcium chloride, 0.1 mM iron sulfate and 15 mM magnesium sulfate.

P-1015 Standardization of Growth Regulators (6-BA; NAA) to improve Rapid In-Vitro Liquid Propagation Medium for **Oxalis Tuberosa** (Oca). J. PEÑAFIEL and R. Estrada. Laboratory of Genetic Resources and Biotechnology, San Marcos State University, P.O. Box 170138, Lima, Peru.

The Andean tuber crops are an important alternative for food production in cold weather highland zones with very poor soil, where often other crops cannot produce. To avoid that genetic erosion decrease this crop's variability, the Laboratory of Genetic Resources and Biotechnology maintains an in-vitro germplasm bank with approximately 1000 entries, which are now being cleaned of virus and electrophoretically evaluated to obtain "characterized" strains. In order for us to provide the improvement programs with large amounts of selected material, the rapid in-vitro propagation methodology was standardized. We first employed the methodology standardized by the International Potato Center (IPC) for potato, but just small thick plants with rosette form growth were obtained. Therefore, we submitted four Oca strains randomly selected to media with a six **Bencil Adenine** (6-BA) and **Naphthalene Acetic Acid** (NAA) concentrations factorial. These plants were incubated for a month at 18 centigrade; 1500 lux; 16-hour day photoperiod. The plants were evaluated on: total nodes obtained from a node sowed, stem height, stem weight, leaf size, roots quantity, number of secondary stems (branches) and vigor (rate stem weight/stem height). We made a two stage selection: in the first stage based on total nodes obtained from a sowed node score, we selected three mediums, one of which was the IPC standardized one. In the second stage, based on stem height, we concluded that the most appropriate medium for fast in-vitro propagation was the one containing MS + 0.05 ppm NAA; 0.01 ppm 6-Ba; 2.0 ppm **Calcium Pantothenate**; 0.4 ppm **Gibberellic Acid**; 3% sucrose; since it presented significantly higher stems than the other selected media. This medium had the additional advantage of inducing the growth of adventitious roots, a result which the other media did not produce. With regard to the rest of the characteristics evaluated the results were very similar for all three media.

P-1016 RAPID MULTIPLICATION OF "THOMPSON SEEDLESS" GRAPEVINE BY *IN VITRO* CULTURE OF SHOOT APICES AND AXILLARY BUDS. AIDA M. ALLAM, DIAA E. EL-RAYES AND M. F. MANSOUR Hort. Dept., College of Agric. Minuriya Univ., Egypt.

This study was carried out at the tissue culture lab. College of Agric., Minouriya Univ., during two successive seasons of 1990 and 1991. Rapid multiplying cultures of "Thompson seedless" grapevines were established from isolated shoot apices and buds on modified Murashige and Skoog (M S) medium supplemented with 5.0 mg/L B A (6-benzylamino purine). The growth rates of these cultures expressed as an increase in shoot length, shoot diameter, and proliferation over an 8-week period were assessed. Addition of GA_3 at a rate of 3.0 ppm during the second stage of development, increased shoot length. The interaction between B A and GA_3 remarkably inhanced proliferation process. Rooted plantlets were produced using indole buteric acid (IBA) at the rate of 1.0 mg/L with half strength medium. The well established plantlets (8 weeks from starting) were kept under humidified condition for a period of two weeks before they were transferred to the green house.

P-1017 In Vitro Hardening of Red Raspberry Plantlets through CO_2 Enrichment, Relative Humidity and Sucrose Reduction

Ribo DENG and Danielle Donnelly. Department of Plant Science, McGill University, Montreal, Canada, H9X 1C0.

Shoots of micropropagated red raspberry (Rubus idaeus L. cv. Comet) were rooted on modified MS media under different sucrose (0, 1 or 5 g/l), CO_2 (ambient or enriched) and relative humidity (RH 100% or 90%). After 4 wks. the rooted plantlets were transferred directly to ambient greenhouse conditions. In vitro hardened photoautotrophic plantlets were obtained from all treatments and they grew successfully without ex vitro acclimatization. Stomata on cultured leaves were not functional regardless of in vitro conditions. Fully functional stomata occurred on the 2nd ex vitro leaves in all cases. In vitro CO_2 enrichment promoted rooting, growth and CO_2 uptake of plantlets and ex vitro transplants. However, this treatment increased stomatal index and apertures with adverse effects on water relation of ex vitro transplants. In vitro RH reduction did not affect rooting, growth or CO_2 uptake of plantlets but improved ex vitro performance of transplants. The RH reduction decreased stomatal apertures, improving water relation of ex vitro transplants. The addition of sucrose in the medium promoted growth but reduced CO_2 uptake of plantlets. The ex vitro performance of transplants was not affected by the inclusion of sucrose in the medium. Thus sucrose may be excluded from red raspberry rooting medium lowering materials cost and reducing contamination.

P-1018 In vitro Production of the Potential Industrial Compound Piquerol from Piqueria trinervia.

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Piqueria trinervia is an annual plant which synthesizes terpene piquerol which has insecticide, molluscicide, allelopathic action and medicinal properties. Average yield of piquerol from dried plants is 10 mg/K. The availability of this resource is decreasing due to the destruction of its habitats. In vitro culture offers the posibility to obtain this plant resource, for its utilization at an industrial scale. The aims of this study were a)to micropropagate P. trinervia and b) to induce in vitro production of piquerol. Seeds of P. trinervia were steril germinated and seedlings were used as explant source. Young and mature leaves, internodes and roots were inoculated into MS basal media supplemented with Kin and 2,4-D (0-3 mg/l). In some experiments NAA and 4 CPA were used. Incubation was carried out at 27 $\pm$ 2°C; 16h at 1200 lux as well as at total darkness. Plant regeneration was attained in internode cultures devoid of hormones or in presence of Kin. Callus production was poor although it was present in most of the treatments. Globular bodies; and hairy root-like structures, conspicuously appeared in all the explants in some of the experi-- ments. These structures developed callus after subcul ture. Presence of piquerol was detected through TLC and GC. The best response was obtained in internode callus growing on MS, 2mg/l Kin at darkness. This callus were used to obtain the kinetic growth curves. It was observed the synthesis of piquerol increases as long as both fresh and dry weight of the callus increases. Piquerol presence was confirmed by NMR.

P-1019 The Effects of Immobilization, Exogenous Enzymes and Precursors on the Production of Artemisinin by Cell Culture of Artemisia annua.

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Artemisinin is an anti-malarial agent isolated from Artemisia annua. Recent reports showed that this compound and its endoperoxide-containing derivatives also have herbicidal activity and are more effective than 2,4-D, glyphosate and other synthetic herbicides. Callus and organ cultures of A. annua maintained in the lab for more than four years, can still produce artemisinin. When various monoterpenes and sesquiterpenes were added into growth media, artemisinin production was increased three to ten times that of the control. This increased amount is equal to or greater than the amount produced by the leaf tissue of naturally grown plants. Addition of glucose oxidase and tyrosinase did not further increase artemisinin production in culture. The results also revealed that the artemisinin can be recovered from both spent liquid and agar media. However, some of the precursors used are toxic. Thus, immobilization techniques were explored for continuously producing artemisinin by cell culture. Our results revealed that polyacrylamide gel alone is not suitable. However, cells immobilized with sodium alginate first, then sandwiched in polyacrylamide gel, can prolong the utilization of the cells for artemisinin production.

P-1020 A Comparison of the Monoterpenoid Constituents of Rosemary Plants Derived from Stem Cuttings and from Leaf Segment Cultures.

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Callus cultures were induced from leaf segments of Rosmarinus officinalis L. 'Lockwood de Forest' cultured on Murashige and Skoog (MS) medium supplemented with 2 mg thidiazuron (TDZ)/liter plus 0.5 mg 3-indole acetic acid (IAA)/liter. The compact green calli were transferred to MS medium supplemented with 4 mg benzyladenine (BA)/liter, where many shoots were formed. Plantlets were transferred to the greenhouse. For oil analysis, fresh samples were taken from both in vitro derived plants and plants produced by traditional cutting propagation, using hexane followed by GC/MS analysis. The oil constituents identified were: α-pinene, camphene, β-pinene, 1,8-cineole, limonene, linalool, camphor, borneol and bornyl acetate. Statistical analysis, using F-tests to test for equality of variances, revealed that the variances were significantly different at p=0.05 for camphene and limonene. However, T-tests indicated that there was no significant difference between the two means. The variance was significantly different at p=0.01 for linalool, and the means were significantly different at p=0.05. Linalool was higher in plants regenerated from callus culture than in the cutting-derived plants. No significant differences were found for oil yield or the other monoterpenes in rosemary oil.

P-1021 Biosynthesis of Lower Terpenes in the Plant Cultured Cells. K.Nabeta, Obihiro Univ. of Agriculture and Vet. Medicine, Obihiro 080, and M.Sakurai, PCC Technol. Mobara 297, Japan.

Biosynthesis of lower terpenes in the cultured cells of plants (*Perilla* sp., *Larix leptolepis* and Pachouli) was examined using whole cells and cell-free preparations with specifically deuterated precursors. Cuparene, the major volatile component of *Perilla frutescens*(Akachirimen) callus have led to a rather complete picture of the labelling patterns by studies using 2,2-$^2H_2$-, 4,4-, 5,5-$^2H_2$ and 6,6,6-$^2H_3$-mevalonate fed to the callus culture. The patterns indicate the posibility of two separate hydride migrations (a double 1,3- and/ or a 1,5-hydride shift) which accompany formation of the cyclopentane ring and the loss of two 5-Hs of mevalonate (protons at C(1) and C(5) of FPP) and one 2-H (proton at C(4) of FPP) to generate the aromatic ring. The formation of α-cedrene in the *L.leptolepis* callus involves a 1,2-hydride shift at C(6) of FPP. Incubation of 6-^{2}H-FPP with the cell-free preparation from *L.leptolepis* callus support this migration. This cell system also generate the deuterated α-cedrene from 1,1-$^2H_2$- 2Z,6E- and 2Z,6Z-FPP but not from 2E-isomers. This finding is opposed to the mechanistic proposals already reported by other workers.The callus tissues of Pachouli(*Pogostemon cabin*) did not accumulate a detectable amount of lower terpenes. When the callus was fed with mevalonate, however, the detectable amount of nerolydol was formed. Synthesis de novo was directly demonstrated by injection of the callus with 6,6,6-$^2H_3$-mevalonate.

P-1022 Development of callus and cell suspension cultures for taxol production. E.R.M. WICKREMESINHE and R.N. Arteca, Department of Horticulture, The Pennsylvania State University, University Park, PA 16802, U.S.A.

Taxol is a cytotoxic diterpene with known antineoplastic activity against both solid tumor and leukemic cell lines. Recent clinical studies show great potential in the treatment of ovarian cancer. The limited supply of taxol has precluded extensive clinical studies against other carcinomas. Callus cultures were initiated from T. media 'Hicksii' stem explants. This particular species and cultivar was selected because of its close resemblance to the native Pacific yew in growth habit, but having a faster growth rate, a higher content of taxol in its stems and needles as compared to the Pacific yew and its greater amenability to tissue culture techniques compared to the other cultivated yews. A fast growing habituated callus line was established from which we have subsequently developed two cell suspension lines (SR-1 and S-3) and one callus line (CR-1). Both cell suspension lines exhibit doubling times ranging from 9-11 days following each subculture. Frequent subculturing was essential for the maintenance of healthy, taxol producing cells. The amount of taxol recovered from the cell suspension medium was less than one third of the amounts found in the cells in the cell line S-3, while in the other (SR-1) the amounts were less than 10%. Taxol yields ranging from 0.01 (cell line S-3) to 0.03% (cell line SR-1) were obtained without elicitation. These amounts are two to three fold higher than the amounts detected in the stems and needles of the original tree. The growth pattern of the callus line (CR-1) was constrained due to the size limitation of the membrane raft system we use for callus production. Maximum taxol production was achieved after 7 weeks in culture. The effects of media components and the use of elicitors will also be presented. Taxol was detected by Reverse phase HPLC analysis, assayed by the microtubule-stabilizing bioassay and finally identified by NMR.

P-1023 Cardiac glycosides in crown galls of Digitalis lanata. M. LUCKNER (1), W. Pinkwart (1), W. Kreis (2), and B. Diettrich (1), Institute of Pharmaceutical Biology, Martin-Luther-University, O-4010 Halle, Germany (1) and Pharmaceutical Institute, University of Tübingen, W-7400 Tübingen, Germany (2)

Wounded D. lanata plants were inoculated with different wild-type strains of Agrobacterium tumefaciens. Crown galls developed at the sites of infection to a size of up to 1 cm within 8 weeks. The incorporation of t-DNA into the crown gall DNA was shown by hybridization experiments using the 1.43 kb BamHI fragment of plasmid pGV 2435 which contains the octopine synthase gene. The cardenolide pattern of crown galls and leaves were examined by TLC and HPLC. They resembled each other. Main cardenolides were lanatoside C and digitoxin. Crown gall tissue cultivated in vitro in a modified Murashige-Skoog nutrient medium showed a much smaller cardenolide content than crown galls attached to the leaves. Thus crown galls in situ may attract cardenolides from the surrounding leaf tissue as they do with nutrients. The cardenolide-modifying enzymes cardenolide glycosyltransferase, cardenolide glucosidase, cardenolide acetyltransferase, and cardenolide acetylase showed different activities in individual crown galls. These differences may be caused by insertion of plasmid DNA into the genome at different positions.

P-1024 Differentiation with Regards to Anthocyanin Production in Grape (*Vitis vinifera*) Cell Suspension. F. CORMIER and C.B. Do. Food R&D Centre. Agriculture Canada. 3600 Casavant Blvd. West, St-Hyacinthe (Québec), Canada J2S 8E3.

Anthocyanin-pigmented cell suspension cultures of grape are mixed populations of producing and non-producing cells. Culture conditions which favour the formation and accumulation of anthocyanins such as medium with high sucrose and low nitrate concentrations and osmotic stress, also favour cytodifferentiation. This translates on one hand, by an increase in the proportion of anthocyanin-pigmented cells (vs non-pigmented cells) and on the other hand, by an increase in the proportion of methylated, more metabolically evolved, anthocyanins. Microscopic observations suggest that stimulating the flavonoid pathway leads primarily to the accumulation of anthocyanins but might also lead to the accumulation of flavonoid-derived tannin bodies.

P-1025 Artemisinin Production by Artemesia annua: Environmental Factors and Transformation. P.J. WEATHERS, R.D. Cheetham, S. Kovacs, A. Hoyen, T. Canty and G. Auger. Department of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA 01609. USA.

The antimalarial compound artemisinin is produced at low levels by the wild type A. annua. To make this compound available to third world patients high yielding plants must be grown under conditions which maximize their capacity for product synthesis. We have examined various tissues from 4 different strains of A. annua and have found the Yugoslavia strain has the best growth characteristics and the highest yield of product. Leaves have more product than stems or roots. Excised roots cultured in shake flasks have a much higher yield (3.2%) than any tissues from intact plants. Environmental stress, such as light, temperature, water and salt, significantly alters product yield. For example, low light increases yield of artemisinin while higher light increases arteannuin ß and arteminisic acid. Transformation efficiencies >70% have been obtained using Agrobacterium. Genetic transformation and environmental conditions which favor biomass accumulation, followed by stress induced product synthesis, may increase product yield to the level where these valuable medicinal compounds can be made available at an affordable price.

P-1026 Tissue Culture and Regeneration of Coastal Dune Grasses. D.M. SELISKAR. Halophyte Biology Laboratory, College of Marine Studies, University of Delaware, Lewes, DE 19958.

Callus culture and regeneration procedures have been developed for two species of coastal dune grasses, *Uniola paniculata* and *Muhlenbergia filipes*, both from the southeastern United States. The protocol for culturing callus tissue of *Ammophila breviligulata*, from the the mid-Atlantic coast, has also been developed. These plants are important stabilizers of the coastal dunes. Over-stabilization of some of the highly managed dunes in highly populated areas has resulted in some pathogen-induced dieout. This along with continuing sea level rise can result in increased coastal erosion. Developing tissue culture systems provides the means to select for possibly resistant plants through somaclonal variation and to establish plant pathogenic nematode inoculum cultures, nematodes being a major threat to *A. breviligulata*. Tissue cultures of the three species were initiated from mature seeds. In the cases of *U. paniculata* and *M. filipes* MS media was supplemented with 0.5 mg l^{-1} of each BA and 2,4-D and 1.0 mg l^{-1} NAA. For *A. breviligulata* MS media was supplemented with 1.0 mg l^{-1} of each IAA and 2,4-D. Regeneration of *U. paniculata* and *M. filipes* was induced by the removal of auxin from the basal medium. In the spring of 1990, regenerated *U. paniculata* plants were planted on dunes in an *A. breviligulata* dieout site in Delaware, north of the northern extent of its normal range. As of August 1991, 90% of the plants were flourishing and many were flowering.

P-1027 Somatic Embryogenesis in the Halophyte *Sporobolus virginicus* (L.) Kunth. J.D. RAO, X. Li and J.L. Gallagher, Halophyte Biology Laboratory, College of Marine Studies, University of Delaware, Lewes, DE 19958

Sporbolus virginicus (L). Kunth is a halophytic grass native to tropical and warm temperature coasts throughout the world. A rhizomatous perennial with erect clums, it occurs along the southeastern coast of the United States as two genetically distinct growth forms, which are designated by their characteristic habitat as "marsh" and "dune". [S. virginicus is of economic value in shoreline stabilization programs and as forage. Callus was induced from immature inflorescences of both ecotypes. Different combinations of media were evaluated for callus induction with the marsh ecotype: MS medium with 2 mg/l 2,4-D was found to be optimum compared to B5 and N6 medium supplemented with 2,4-D. Immature inflorenscences produced friable, yellow callus both on the MS and N6 media. Cultures displayed compact, nodular embryogenic calli after transferring to MS medium with 2,4-D 0.5 mg/l, BAP 0.5 mg/l, and NAA 1 mg/l and 5% coconut water. Callus cultures with globular structures of embryogenic callus were first apparent after about one month of culture when transferred onto MS medium with BAP, NAA and 5% coconut water. An average of 15-20 shoots per gram of callus was produced. The suspension culture of embryogenic cells was established from immature inflorescences of the marsh form of Sporobolus virgincus. Embryogenic callus was placed into liquid MS medium with 2,4-D where it readlily dissociated to form suspension cultures. The primary suspension cultures contained aggregates of small, densely cytoplasmic cells.

P-1028 Preparation of Protoplasts of Halophytes and the Evaluation of Salinity Tolerance as a Natural Fusion Marker in *Kosteletskya virginica* and *Sporobolus virginicus* (L.) Kunth. XIANGGAN LI, J.D. Rao and J.L. Gallagher, Halophyte Biology Laboratory, College of Marine Studies, University of Delaware, Lewes, DE 19958, USA

Kosteletskya virginica is a perennial herbaceous dicot of the family Malvaceae. It can tolerate 70% strength seawater with good yields of up to 1300 lbs of seed per acre. *Sporobolus virginicus* in the Poaceae, can tolerate up to 80 PPT salinity (sea water-35PPT) when grown as a forage crop under saline irrigation. Suspension culture cells of *K. virginica* and friable embryogenic callus of *S. virginicus* have been used successfully to isolate protoplasts with yields of up to several million protoplasts per gram fresh weight of cells. Maximum yields of *K. virginica* protoplasts have been obtained by incubating cells in high concentration of enzymes (2% RS cellulase, 0.5% macerozyme and 0.1% pectinase) at 21°C for 12 hours and in low concentration at 28°C for 3 hours. For *S.virginicus* maximum yields were obtained in high concentration at 21°C for 6 hours and in low concentration (1% RS cellulase, 0.5% macerozyme, and 0.05% pectinase) at 21°C for 12 hours. Cell wall regeneration and cell division have been observed in protoplast culture of *K. virginica* in 11% mannitol medium. The change in viability with time was the same for control and salinity stressed cultures. This indicates that protoplasts of halophytes retain the capability to tolerate salt. Therefore salt tolerance may be useful as a marker for selecting fusion products involving halophytes and glycophytes.

P-1029 Somatic Embryogenesis on Immature Cotyledons of Watermelon. M.E. COMPTON and D.J. Gray. CFREC/IFAS, University of Florida, 5336 University Ave, Leesburg, FL 34748.

Cotyledon explants from immature embryos of 4 watermelon genotypes were plated on MS medium with 30 g/l sucrose, 1 g/l myo-inositol, B_5 vitamins, 7 g/l T.C. agar and 0.5 μM benzyladenine (BA) or thidiazuron (TDZ) combined with 2,4-D at three concentrations (10, 20 and 40μM). Explants were incubated on medium with plant growth regulators (PGRs) for three weeks in the dark before transfer to medium without PGRs and 16h photoperiod. Somatic embryos were observed on cotyledon explants of 'Allsweet', 'Crimson Sweet', FLAS87 Gate and 'Minilee' three to four weeks later. Some embryos formed well developed cotyledons and hypocotyls; however, a majority of the embryos were abnormal with enlarged hypocotyls and poorly formed cotyledons. Embryo formation was greatest on medium with 0.5 μM TDZ and 10 or 20 μM 2,4-D. Somatic embryos that germinated on MS medium without PGRs (4 weeks) were transferred to soil and gradually acclimatized to the ambient environment (3 weeks). Somatic embryo-derived plants were transferred to the field where they formed fertile male and female flowers that produced normal fruit. To our knowledge this is the first report of the regeneration of fertile plants from watermelon somatic embryos.

P-1030 Inheritance of Green Plant Production in Wheat Anther Culture. H. ZHOU and C.K. KONZAK Dept of Crop and Soil Sciences, Washington State University, Pullman, WA 99164-6420

The low percentage of green plants from anther culture is a major restriction of haploid production for cereal crops. The objective of this study was to determine the inheritance of green plant percentage in wheat anther culture. Reciprocal crosses and reciprocal backcrosses were made between two spring wheat genotypes (Chris and Yecora Rojo) that produce high percentage of green plants and two genotypes (Edwall and WA 7176) that produce low percentage of green plants. Anthers from the parents, reciprocal crosses and reciprocal backcrosses were cultured to determine the relative importance of nuclear gene in the control of green plant production. The percentage of green plants of the F1's were intermediate between their parents. No significant differences were detected in all reciprocal crosses whereas reciprocal backcrosses shifted the percentage of green plants toward their male parents. These results suggest that the percentage of green plants is under the control of nuclear genes and these genes are codominant. Significant differences in green plant production among individual plants within genotypes were detected, indicating a potential improvement of anther culture response by in vitro pre-screening.

P-1031 The Effect of Epi-24 Brassinolide on Callus and Root Formation in Saskatoon (Amelanchier alnifolia Nutt.) Microcuttings. K. PRUSKI, T. Lewis and M. Mirza. Alberta Tree Nursery and Horticulture Centre, R.R. #6, Edmonton, Alberta, CANADA, T5B 4K3

There is very little information on application of brassinosteroids in tissue culture propagation. The effects of several types of brassinosteroids on mature plants were described by Chen (1983, Chemistry in Canada 35:13-16). In the present study epi-24 brassinolide (Grow Tec Ltd., Nisku, Alberta, Canada) was incorporated into the rooting medium. Saskatoon microcuttings about (2 cm) produced in vitro were individually placed in test tubes (one microcutting per test tube) on ½ MS salts containing 20 g/L of sucrose and various concentrations of epi-24 brassinolide (from 0.05 mg/L to 5 mg/L ± 0.5 mg/L of IBA. Cultures were incubated for 4 weeks in the growth room at 24°C with 16 hours photoperiod at 3000 lux. Concentrations of epi-24 higher than 1 mg/L were toxic to cultures. Very little rooting was observed when epi-24 (concentrations 0.1 - 0.3 mg/L) was used alone in the medium. If used in combination with 0.5 mg/L of IBA, 100% rooting was observed and no callus formation at the base of microcuttings comparing to heavy callusing on medium with IBA alone. Some of the roots, however, were slightly twisted but in general roots were longer on media with epi-24 brassinolide (concentrations 0.1 - 0.3 mg/L) than on media with IBA alone.

P-1032 Desiccation and Cryopreservation of Embryo Axes of Quercus sp. V.C. PENCE, Center for Reproduction of Endangered Wildlife, Cincinnati Zoo and Botanical Garden, 3400 Vine St., Cincinnati, OH 45220

The effects of varying amounts of desiccation and subsequent freezing in liquid nitrogen were tested on embryo axes of Quercus. Surface sterilized axes were aseptically desiccated under the air flow of a laminar flow hood, and removed at intervals of 15 min. At each harvest, axes were either cultured directly on Woody Plant medium; fast frozen in liquid nitrogen before thawing and culture; or used for moisture determination. Survival, root initiation and shoot initiation were followed. Dried, nonfrozen embryos showed good survival at all harvests, up to 90 minutes, or 11-15% moisture. Root initiation also did not appear to be affected by desiccation, whereas shoot initiation was sporadic and did not follow a visible pattern. Frozen axes did not survive without 15 or 30 min desiccation. Below about 25% moisture, the number of axes surviving was equivalent to that of nonfrozen embryos. Shoot initiation from frozen axes was equivalent to or better than from nonfrozen embryos, once moisture levels were reduced to approximately 20%. Root initiation from frozen embryos was equivalent to that of nonfrozen embryos when moisture levels were less than about 17%. When total embryo length was analyzed, freezing either had no effect or was inhibitory, depending on the species. These results suggest that oak embryos can survive desiccated freezing and can subsequently initiate roots and shoots, but that growth may be reduced in some cases by such treatment. Culture conditions are also needed to optimize full germination of Quercus axes in vitro.

P-1033 High Frequency Adventitious Shoot Regeneration and Transient Gene Expression in Pea Cotyledon Explants.
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Immature pea cotyledons were cultured on MS medium containing 0-4 mg/l 6-benzylaminopurine (BAP) and 0-8 mg/l α-naphthaleneacetic acid (NAA) or 0-8 mg/l indole-butyric acid (IBA). Prolific shoot regeneration occurred at the wound site following an initial callus growth on a medium containing 0.5 mg/l BAP and 4 mg/l NAA (1). Cotyledon explants proximal to the embryonic axis had the highest regeneration potential however, presence of an embryonic axis inhibited adventitious shoot regeneration. Addition of silver nitrate to the medium did not increase the number of regenerated shoots but resulted in longer shoots with well developed tendrils and large stipules which had a reduced rooting capacity. Regenerated shoots rooted in half strength MS medium containing 1 mg/l IBA and further established well in compost. In order to identify a suitable gene transfer system for pea, plasmid constructs containing the GUS (ß-glucuronidase) gene under the control of the CaMV 35S promoter were used in microprojectile experiments. Cotyledon explants were bombarded with DNA-coated tungsten particles and transient GUS gene expression was observed 2-14 days post-firing.

1. Özcan et al (1992) Plant Cell Reports (in press).

P-1034 Studies on Tissue Culture of *Eucommia ulmoides* Oliv.
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Eucommia ulmoides Oliv. distributed in China belongs to Eucommiaceae, known as a medicianal tree. The leaves and barks of this tree are used for a drink. Chinese literatures refer to Eucommiae cortex as a diuretic etc. At present in China, it seems like that this species has been decreasing in number because a large scale deforestation. Therefore the propagation *in vitro* and the extraction of effective components of *E. ulmoides* were investigated. The germination rate of seeds showed from 7% to 10%, but the immature embryoid germination *in vitro* was increased by an addition of cytokinin. And then, multipul shooting and rooting of hypocotyl cultured were succeeded. In stem-culture many shoots were induced by using WP and B5 mediums. Generally, it is said that the cultured cells do not metabolize the medicinal components etc., but the pinoresinol-di-0-β-D-glucoside, which has the remarkable effect as a hypotensive drug, was detected in the cultured cells.
Moreover, the characteristics and the components of the cultured cells in *E. ulmoides* were discussed.

P-1035 In Vitro Shoot Formation of Cacti Species in Response to Cytokinins and Auxin.
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Commercial propagation of cacti species is usually done by using seeds or cuttings. However, some growers still collect many of these species from their natural habitat, so that several species are in danger of extinction. Development of cacti shoots in vitro can be accomplished, but success depends on the specie and the composition of the culture medium. The objetive of this research was to determine the in vitro shoot formation of cacti species in response to cytokinins and auxin. Explants consisting of shoot and callus segments were taken from cultures originated from in vitro-grown seedlings of Pelecyphora pseudopectinata, Neolloydia lophophoroides and other cacti species, and then were plated on a Murashige and Skoog medium containing kinetin (KN) or benzyladenine (BA) at 0, 0.2, 0.4, 0.8, 1.6 and 3.2 mg/liter +/- 0.2 mg/liter indoleacetic acid (IAA). When KN was present on the medium, the highest number of shoots per culture was obtained with 0.8 mg/liter -IAA (11.2 shoots) and with 3.2 mg/liter + IAA (13.2 shoots). On the other hand, when BA was used, the number of shoots per culture was greatest at 0.2 mg/liter - IAA (16 shoots) and + IAA (18 shoots). Shoots were much larger at the lower concentrations of both cytokinins, with IAA having little effect. These results indicate that the in vitro shoot formation of cacti species is best induced with low levels of BA (0.2 mg/liter) without IAA.

P-1036 In Vitro Regeneration of Grain Legumes: A Novel Approach.
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Plant cells possess a unique capacity to develop by regeneration into whole multicellular organisms. This capacity is expressed in vitro in cultures of tissues and cells where competent cells display their inherent potential of organogenesis or somatic embryogenesis. Typically, regeneration of organs or somatic embryos is accomplished in two steps: the isolation of appropriate explants and their culture on a medium supplemented with nutrients and growth regulators. However, conventional explant culture methods have produced rather limited success in regenerating large-seeded grain legumes. We have developed a novel approach for inducing organogenesis and somatic embryogenesis in many legume species including pea, peanut, bean, and lentil. In this method, mature seeds are germinated in the presence of 6-benzylaminopurine or thidiazuron, a substituted phenylurea known to elicit cytokinin-like activity. *De novo* differentiation of shoots or somatic embryos occurred from different regions of morphologically intact seedlings and the regenerants were capable of developing into mature plants. The frequency of regeneration from intact seedlings was higher than that obtained with explant culture. The whole process of the induction and expression of organogenesis and somatic embryogenesis involves a single step of seed culture which significantly reduces the number of cultural manipulations associated with the optimization of isolation and culture of explants. High frequency of direct regeneration of plants from intact seedlings demonstrates the significance of morphological integrity of the source plant on *de novo* cell differentiation. The procedure was applicable, in principle, to many non-leguminous species also and is expected to be useful in producing transgenic plants and in studying plant development in vitro.

P-1037 Embryogenic Cultures from Various Explants of Soybean. P.S. KAHLON and S.M. Bhatti. Cooperative Agriculture Research Program Tennessee State University, Nashville, Tennessee 37209-1561.

Most studies on somatic embryogenesis in soybean have been carried out using cotyledons of early stage immature embryos. Methods employed have included using high levels of 2,4-Dichlorophenoxy-acetic acid (2,4-D) or Naphthalene acetic acid (NAA). We are investigating the development of somatic embryos using mature cotyledons, leaves, hypocotyls and root sections of two cultivars of soybean. Seeds of cultivars Weber and Pella were allowed to germinate in test tubes for 2-3 weeks. At this time, the various explants mentioned above were plated on Murashige-Skoog (MS) and L6 media containing 2 or 4 mg/l of 2,4-D and solidified with 0.8% agar. Within three weeks, smooth shiny callus formed on leaf sections cultured on L6 media. At the end of the six week period, a large number of embryos were produced from this callus. However, a lower frequency of embryos were observed when MS media was used. The root sections also produced embryos at a higher frequency on L6 media than on MS media. No significant difference was observed between the two media when cotyledon and hypocotyl explants were used.

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P-1038 Artificial Seeds of Conifers. S.M. ATTREE, and L.C. Fowke. Dept of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, S7N OWO, Canada.

Abscisic acid (ABA) and water stress are important for maintaining embryo maturation, and drying occurs naturally during seed development. High levels of ABA were vital for maturation of Picea glauca somatic embryos; however, the inclusion of a moisture stressing treatment, effected by permeating osmotica (e.g., sucrose), with ABA inhibited development. In contrast, a moisture stressing treatment effected by non permeating compounds (e.g. polyethylene glycol [PEG]) with ABA promoted maturation. PEG stimulated maturation frequencies threefold, inhibited precocious germination, decreased moisture contents by 25%, and increased dry weights correspondingly. The latter changes were found to be due to increased levels of storage reserves (both triacylglycerol [TAG] and storage proteins). The TAG fatty acid composition resembled that of zygotic embryos. Conditions that increased storage reserves promoted desiccation tolerance; furthermore, following maturation with PEG, subsequent embryo desiccation was vital to permit normal plantlet development. A transmission electron microscope study showed that the structure and distribution of lipid and protein bodies, and degree of embryo development, were similar to mature zygotic embryos. The somatic embryos underwent vigorous, high frequency plantlet conversion. Desiccated somatic embryos survived freezing both at -20 °C, and in liquid nitrogen. Plantlet conversion was also obtained from somatic embryos following desiccation and encapsulation in non-hydrated water soluble capsules.

P-1039 Tomato Fruit Aroma Compounds and Sugars: Further Evidence of the Altered Developmental Progaram of In Vitro-Cultured Tomato Calyx. BETTY K. ISHIDA, ELIZABETH A. BALDWIN,* RON G. BUTTERY, LOUISA C. LING, AND GEORGE H. ROBERTSON. Western Regional Research Center, U.S. Department of Agriculture, ARS, 800 Buchanan Street, Albany, CA 94710, and *U.S.D.A. Citrus and Subtropical Products Laboratory, Winter Haven, FL 33883.

A unique system to study fruit ripening in vitro has been developed in this laboratory (Ishida, Betty K., The Plant Cell 3: 219-223, 1991). Ovulary cultures were initiated from VFNT Cherry tomato flowers. Tomato fruit ripened after 6 to 7 weeks, but the calyces unexpectedly swelled and gradually became red and succulent. The following changes occurred in both ripening fruit and ripening calyx: increased ethylene production, increased 1-aminocyclopropane-1-carboxylic acid (ACC) content, increased mRNA of polygalacturonase (which in tomato is present in large amounts only in ripening fruit), and disruptive changes in the cell wall as shown by electron microscopy. These calyces also ripened when cultured in the absence of fruit. Recent studies show that volatile compounds that are important to tomato flavor increase in ripening calyx as they do in ripening fruit. Changes in sugar content were also similar. In attempts to induce ripening-related changes in tomato callus cultures, increased lycopene synthesis was obtained, but not increases in volatile tomato flavor compounds. These data provide further evidence that the developmental program of the leaf-like calyx of this tomato was actually altered to that of fruit tissue.

P-1040 Optimization of plant regeneration from callus cultures of Gladiolus. K. KAMO and M. Dorak. Florist and Nursery Crops, U.S.D.A., Beltsville, MD 20705-2350

The number of plants regenerated from callus cultures of the Gladiolus genotype Jenny Lee was determined for optimization of regeneration. Callus was initiated from either cormel slices or in vitro plantlets that were cultured on solidified Murashige and Skoog's basal salts medium supplemented with vitamins, sucrose and various concentrations of auxin (α-naphthaleneacetic acid, dicamba, 2,4-dichorophenoxyacetic acid) or cytokinin (N^6-(2-isopentenyl) adenosine, 6-benzylaminopurine, kinetin). The maximum response to auxin by cormel slice explants was 55-65% of the cormel slices cultured on 0.5 or 1 mg/l 2,4-D formed embryogenic callus. Embryogenic callus was formed by 83-100% of the in vitro plantlets when cultured on 20 mg/l NAA, 2 mg/l dicamba, or 2 mg/l 2,4-D. Cormel slices cultured on every cytokinin tested formed callus and rapidly regenerated plantlets within 3-4 months after the cormel slices were initially cultured. There was an average of 22 or 12 plantlets regenerated/cormel slice for cormel slices that formed callus when cultured on 1 mg/l BAP or 10 mg/l iPA respectively. Although there were more plantlets regenerated from cormel slices cultured on 1 mg/l BAP than 10 mg/l iPA, 68% of the cormel slices formed callus and regenerated plantlets on iPA compared to only 35% on BAP.

P-1041 Effects of Cytokinins, Genotype and Other Factors on Somatic Embryogenesis From Cotyledons of *Cucumis melo*. D.J. GRAY, D.W. McColley and M.E. Compton. CFREC, IFAS, University of Florida, 5336 University Ave., Leesburg, FL 34748.

Somatic embryogenesis was obtained from mature seed cotyledon bases of *Cucumis melo* (muskmelon) after induction on solidified MS medium containing 5 mg/l 2,4-D and a test cytokinin, followed by transfer to medium lacking growth regulators. The effects of cytokinin type and amount in the induction medium, sucrose concentration in both media, and induction period on somatic embryo number and quality were investigated. Of the cytokinins benzyladenine, kinetin, thidiazuron (TDZ) and 2iP, each tested at 0.1, 1 and 5 mg/l, 0.1 mg/l TDZ was superior with 71% of explants producing an average of 14 somatic embryos each. Three percent sucrose in both induction and basal media was better than higher or lower concentrations. Cotyledons exposed to induction medium for 1 or 2 weeks produced more somatic embryos than those cultured for 0, 3 or 4 weeks. Comparison of 50 varieties with this improved protocol demonstrated distinct genotypic differences in response. With select lines, 100% of cotyledon explants produced at least one somatic embryo with an average of 11 embryos per cotyledon. Over 40 embryos per cotyledon were routinely obtained. Somatic embryos were green and passed through recognizable embryonic stages. Although abnormal, precociously germinating embryos occurred, morphologically correct embryos were common. Fertile plants of normal appearance were obtained from both embryo types.

P-1042 Plant Regeneration and Somaclonal Variation in the Leaf Segment Culture of *Rumex acetosa*. J. W. BANG and M. K. LEE. Department of Biology, College of Natural Sciences, Chungnam National University, Daejeon, 305-764, KOREA

Leaf segments of the dioecious plant *Rumex acetosa* were cultured *in vitro*. Whole plants were regenerated through callus culture. Somaclonal variations between male and female were also studied. Calli were developed on the modified MS medium containing 2mg/l of 2,4-D and 0.1mg/l of kinetin and shoots were induced and multiplicated on the medium containing 2.2mg/l of BAP and 0.1mg/l of IAA. Roots were developed on the medium supplemented with 2mg/l of IBA.

The frequency of shoot development from the callus and the growth rate of regenerated shoots in male were higher than those in female. The numbers of shoots developed from a callus were 10-20 in male and 5-6 in female. The durations required in root development from the shoot were 2 weeks in male and 8 weeks in female. Chromosomal variations were found in the callus cells. Doubling and loss of one or two chromosomes were found in the male callus cells. Considerable chromosomal aberration were detected in the female callus cells. The callus cells of female contained 39 - 79 chromosomes and normal chromosome complement was not found at all. It was suggested that chromosomal stability in the cultured cells of male was higher than that in female. Polyploidization was the common phenomenon in the callus cells. Regenerated female plants showed normal chromosome complement, while all the male regenerants were tetraploids.

P-1043 Triploid Plantlets From The Endosperm Culture of Some Euphorbiaceae. N. SYED ABBAS ALI, Department of Botany, University of Delhi, Delhi-110007, India

Triploid plants are more vigorous than their diploid counterparts. Besides their fore-most use in the hypridization programmes, they are also used for the augumentation of reservoirs of germ plasm. The immature and mature fatty endosperm of Mallotus philippensis (3n = 33) and Emblica officinalis (3n = 147) was cultured on MS enriched with various phytohormones. Only the mature endosperm of M. philippensis proliferated in 3 weeks (wk), on MS + 2,4-D (1 mg/l) + KN (0.5 mg/l). The callus became compact and nodulated after another 6 wk on MS + NAA (0.5 mg/l) + BAP (1 mg/l). When subjected to chilling at 12C for 19h in dark, these nodules differentiated into multiple leafy shoots in 80% cultures on MS + IAA (0.5 mg/l) + BAP (2 mg/l) + Glutamine (50 mg/l) within 17 wk. In E. officinalis, a continuously growing active callus developed on MS + 2,4-D (1 mg/l) + KN (1 mg/l) after 3 wk under continuous light at 25C. The subcultured callus, on MS + BAP (0.2 mg/l) + IAA (0.1 mg/l) resulted in the production of shoot buds, shoots and embryoids, under diffuse light after 6 wk. These somatic embryoids differentiated on the same medium into triploid plantlets (3n = 147) in 73% cultures. High frequency of differentiation through embryogenesis was observed when GA_3 (0.5 mg/l) was incorporated into MS + IAA (0.1 mg/l) + BAP (0.2 mg/l). The in vitro-raised plants were successfully transplanted to field. Efforts are under way to obtain hexaploid plantlets.

P-1044 Embryogenesis from Non-Juvenile Norway Spruce (Picea abies). R.J. WESTCOTT. Unilever Research, Colworth House, Sharnbrook, Bedford MK44 1LQ, England.

The most serious constraint to the exploitation of biotechnology for improvement in forestry is related to the physiological changes within a tree related to aging. For Picea abies this includes low frequency of rooting, loss of growth capacity and plagiotropic growth of cuttings taken from shoots collected on trees older than about four years. Selections of elite individuals cannot be made with any certainty until seedlings are much older (8-15 years).

In tissue culture both organogenesis and somatic embryogenesis are routine starting with zygotic embryo explants or very young cotyledons from seedlings up to 30 days old. A method has been developed which induces somatic embryogenesis in callus derived from bud and needle explants of young trees (7 years old). Attempts are being made to repeat the method with callus from mature (flowering age) trees and obtain further development to plantlets.

P-1045 Transformation of barley (Hordeum vulgare L.) microspores: K.J. KASHA, A. Ziauddin and E. Simion. Crop Science Dept., University of Guelph, Guelph, Ontario, N1G 2W1 CANADA.

Initial phases of this project involved developing a system where microspores in the early stages of division could be bombarded using the Biolistic™ particle gun and then cultured to obtain high frequencies of green plant regeneration. Use of solid media, filter paper and a membrane were all essential as a support system during bombardment and subsequent transfers. This system can be routinely used for cultivars Igri, Elrose and Sabarlis. We have bombarded microspores of different ages after culture initiation and the most promising results have been with microspores that are 5 days old or younger (including those freshly isolated). For selecting potential transformants we have been using two different plasmids. One contains the anthocyanin (ant) pigment and the other contains both selectable marker for the herbicide BASTA (the bar gene) as well as GUS. The amount and degree of the anthocyanin expression has been found to vary considerably. It is often expressed in the scutellar regions of the developing embryo and in the older leaves of the developing plantlet. Use of 3 mg/l BASTA was most effective for selection purposes. A number of plants have survived 6-8 weeks of subculturing on selection media. A number of experiments are currently in progress and the results from these will be presented. A number of potential transformants have been produced which need to be tested further to obtain conclusive evidence of transformation. (Research support from NSERC, OMAF and Monsanto is gratefully acknowledge).

P-1046 Development of the Particle Inflow Gun for Studies on Genetic Transformation of Plant Cells. J.J. FINER, P. Vain, M.W. Jones, M.D. McMullen[1]. Department of Agronomy, The Ohio State University, Wooster, OH 44691, USA, [1]USDA/Agricultural Research Service.

A simple and inexpensive particle bombardment device was constructed for delivery of DNA to plant cells. The Particle Inflow Gun (PIG) device is based on acceleration of DNA-coated 1.0μm tungsten particles using pressurized helium in combination with a partial vacuum. The particles are accelerated directly in a helium stream rather than being supported by a macrocarrier. The absence of a macrocarrier facilitated the use of low helium pressures (40-80 PSI) for particle acceleration. Bombardment parameters were partially optimized using maize embryogenic suspension cultures and cowpea leaves as target tissues and transient expression assays of a ß-glucuronidase gene containing the maize *Sh1* intron or no intron respectively. Factors such as helium pressure, flight distance, and use of baffles were evaluated. High levels of transient expression of the ß-glucuronidase gene were obtained following bombardment of embryogenic suspension cultures of corn and soybean, and leaf tissue of cowpea. Thousands of blue foci were obtained with all of these tissues for each bombardment. Filtration and plating of corn cells prior to bombardment resulted in a large increase in transient expression levels with these cells. Stable transformation of embryogenic tissue of soybean and corn has been obtained using this bombardment apparatus.

P-1047 Bacteria and Yeast Cells as Projectiles for Biolistic Transformation of Plants. J.A. RUSSELL, J.L. Rasmussen, M.K. Roy, and J.C. Sanford. Cornell Univ., Plant Science Center and Dept. of Hort. Sci., Geneva, NY 14456; and Dept.Biology, SUNY - Plattsburgh, NY.

Bacteria and yeast projectiles could be a useful vehicle to deliver DNA into plants. To test this hypothesis, we bombarded tobacco cell suspensions with *E. coli* or yeast (*S. cerevisiae*) cells that harbored a plasmid containing the GUS and NPTII genes. Corn cells were bombarded with *E. coli* containing the B and C1 genes (anthocyanin production). Bacteria and yeast were grown to mid-log phase, were concentrated to an OD_{600} of 10 - 20, were dried in 10 µl aliquots onto Kapton discs, and were accelerated in a helium-driven biolistic device. The average number of transient transformants (ie. blue or red cells) was 5 to 80 per bombarded plate with *E. coli* and 0 - 3 with yeast. When 1 µm naked tungsten particles were mixed with the *E. coli cells*, the number of transient transformants increased to 100 - 2000 per plate. To eliminate background GUS activity, it was necessary to treat the *E. coli* with 1% phenol for 30 minutes prior to bombardment. Stable transformants have not yet been recovered, possibly because of excessive plant cell injury from these relatively large microprojectiles. *E. coli* projectiles can now be used for transient gene expression experiments without time consuming DNA purification. With further improvements, bacteria and yeast projectiles might be useful for delivery of very high molecular weight DNA into plants.

P-1048 A Monocot. Wound-Induced Promoter (AoPR1) is Specifically Expressed in Target Cells for Plant Transformation. S. FIREK, S. Özcan, S. Warner & J. Draper, Botany Department, Leicester University, University Road, Leicester LE1 7RH, U.K.

A wound-induced promoter (AoPR1) isolated from *Asparagus officinalis* was shown by GUS reporter gene analysis to be active during callus formation in tissue cultured leaves from transgenic tobacco plants. This was investigated further by use of an intron-containing GUS gene which allowed the expression of the AoPR1 promoter to be monitored during the very early stages of *Agrobacterium*-mediated transformation. GUS expression were found to be localised to those cell types which are targets for plant transformation. Interestingly, unlike other promoters commonly used to drive expression of marker genes during transformation e.g. pNos and CaMV 35S, the AoPR1 promoter is expressed at very low levels in the leaves and roots of the mature plant. This property is perhaps highly desirable in the context of future commercially produced transgenic plants since the presence of high levels of marker gene products in foodstuffs may be unacceptable. To this end a plant transformation vector was constructed in which Npt-II expression was placed under the control of the AoPR1 promoter. This construct was then used in transformation experiments which resulted in the successful production of a number of transgenic tobacco plants. In these plants the presence of marker gene proteins was undetectable when compared to the very high levels found in control plants. Preliminary experiments have also shown that the AoPR1-Npt-II construct can be used to transform several other plant species including *Brassica napus* and *Arabidopsis thaliana*.

P-1049 A Simple Direct Technique of Transformation in Sunflower.
A. ESPINASSE. South Dakota State University, Plant Science Dept., Brookings, SD 57007

Immunologic, histochemical and molecular evidences suggest that transgenic plants of sunflower, Helianthus annuus L., were produced through microinjection of alien DNA at defined times after pollination into fertilized ovules of sunflower grown in vitro. Plasmid pBI221 (CloneTech), a 3Kb B-Glucuronidase (GUS) gene construct on a HindIII-EcoR1 fragment of pUC19, was used as the alien DNA. Rates of embryo and plant recovery from the in vitro grown ovules varied with the genotype and the age of the ovules. Older ovules produced more embryos and eventually more plants. Effects of the medium composition could not be detected. Averaged across genotypes, media, and ovule ages, 2% of the cultured ovules produced blooming plants and 30% of these plants were found to be transgenic. Amounts of plasmid pBI221 injected did not affect transformation efficiency measured in terms of percentage of transgenic plants, but could affect the frequency of the alien gene introgression. Most of the transgenic plants only integrated one single copy. Progenies of the confirmed transgenic plants were tested using a PCR technique. One fourth were found to have inherited the GUS gene as expected for single copy introgression. The advantages of the developed technique are its ease of implementation, low cost, good efficiency, and applicability to several genotypes.

P-1050 Negative and negative-positive transformation marker combinations for homologous gene replacement in Arabidopsis and tobacco.
L.MÁRTON and M.Czakó. Dept.of Biology, Univ.of South Carolina, Columbia,SC 29208.

Site specific transformation events based on homologous recombination can be directly selected by marker restoration in the target area. If the functional restoration is not possible (lack of selectable or visible markers), an indirect approach can be applied, the use of flanking negative selection marker(s) linked to the homologousregion of the donor DNA.
We have developed for plant gene targeting:
-a conditionally lethal marker for negative selection from the human Herpes Simplex Virus thymidine kinase gene coding region,
-a lethal marker from the diphtheria toxin A fragment coding region.
A new positive, visible and vital marker from Pseudomonas indigo genes is being developed. Arabidopsis and tobacco cultures were transformed with Agrobacterium, carrying different binary vector constructions. Positive and negative selection markers were combined in model experiments and the proper selection conditions were developed. As it has been demonstrated, the HSV-tk marker allows negative selection in a tissue specific manner, while the DTx-A marker allows a more uniform radical negative selection, but still does not exclude transformation events where the DTxA gene is not involved. It has been tested with weaker and stronger promoters.

P-1051 Optimization of Microprotoplast System for Limited Gene Transfer in Plants.
K.S. RAMULU, H.A. Verhoeven, P. Dijkhuis and J. Blaas. Department of Cell Biology, DLO-Centre for Plant Breeding and Reproduction Research CPRO, P.O. Box 16, 6700 AA Wageningen, The Netherlands.

In recent years, much attention is being focussed on the identification and mapping of plant genes which code for economically important characters, because only the identified and biochemically well characterized genes can be transferred through DNA transformation techniques. Further, several desirable traits, like drought or frost tolerance, disease resistance, etc. are encoded by multiple genes which are either scattered throughout the genome, or clustered within blocks. Therefore, these traits are not yet amenable for manipulation by the presently available transformation techniques. In this regard, new approaches are highly desirable. Fusion of microprotoplasts which contain only one or a few specific chromosomes (in interphase) surrounded by nuclear membrane and a small rim of cytoplasm, offers the possibility for transfer of unidentified genes, or those controlling complex polygenic traits across taxonomic borders. We have recently optimized the 'microprotoplast system' in various plant species. The system involves mass induction of micronucleated cells through treatments with a spindle toxin, such as amiprophos-methyl, isolation of microprotoplasts on an iso-osmotic gradient of Percoll using ultracentrifugation, and purification and enrichment of sub-diploid fraction of microprotoplasts. The enriched fraction of microprotoplasts containing one or a few chromosomes are transferred from the donor lines to the recipient lines through fusion. Since the donor lines carry selectable markers (Km^r), heterokaryons are selected on a conditioned medium. The production of microcell hybrids in potato, tomato and Nicotiana and the application of 'microprotoplast system' for limited gene transfer will be discussed.

P-1052 H.D. Quemada[1], D.M. Tricoli[1], R.Z. Deng[1], P.F. Russell[1], J.R. McMaster[1], M.L. Boeshore[1], J.F. REYNOLDS[1], N.E. Bieber[1], D.W. Groff[2], K. Hadden[3], B. Moraghan[3], A. May[3], J.P. Hubbard[3]. [1]Experimental Plant Genetics, The Upjohn Company, Kalamazoo, MI 49001, [2]Southeast Breeding Station, Asgrow Seed Company, Tifton, GA 31794, [3]Plant Pathology Research Center, Asgrow Seed Company, San Juan Bautista, CA 95045

Genetic Engineering of Multiple Virus Resistance in Plants: Data from Field Trials.

The commercial production of cantaloupe is compromised by virus infections. There are at least seven plant viruses that are known to infect curcurbit production fields; the most common are cucumber mosaic virus (CMV), papaya ringspot virus (PRV), watermelon mosaic virus 2 (WMV2) and zucchini yellow mosaic virus (ZYMV). These viruses typically infect fields in combination. Horticulturally acceptable varieties which have genetic resistance to these viruses are not available in cantaloupe. Genetic engineering offers a means of developing cucurbit hybrids which exhibit protection from virus infection without altering the plants' horticultural characteristics. It has been demonstrated that expression of viral coat protein genes in transgenic plants protects these plants from infection by the virus from which the coat protein gene was derived. We have applied this observation to cantaloupe plants by employing *A. tumefaciens* mediated transformation to introduce plant expressible CMV, WMV2, ZYMV or PRV, gene(s) into the genome of selected Asgrow inbred lines.

Plant tissues were transformed by co-cultivation with disarmed *Agrobacterium tumefaciens*. These strains contained a binary plasmid containing the selectable marker neomycin phosphotransferase (NPT II), a viral coat protein gene, and the scorable marker ß-glucuronidase (GUS). Regenerated transformed plants were identified by (1) their ability to root on 200 mg/l kanamycin, (2) the presence of the scorable GUS marker, (3) positive ELISA for NPT II and (4) Southern analysis using NPT II and coat protein gene sequences as probes.

Transgenic R_1 seed was produced by self or cross pollinating established greenhouse R_0 plants. Segregating R_1 seed was used for field test at multiple U.S. locations. Our results show protection against viruses under a variety of environmental and disease pressures.

P-1053 ETHYLENE, CO_2 AND O_2 CHANGES DURING SOMATIC EMBRYO DEVELOPMENT IN LIQUID CULTURES OF *Ipomoea batatas*. M.E. BIENIEK, R.C. Harrell and D.J. Cantliffe. (MEB, DJC) Vegetable Crops Dept. (RCH) Agricultural Engineering Dept., IFAS, University of Florida, Gainesville, FL 32611 USA

High frequency somatic embryogenesis is routinely obtained in suspension cultures of *Ipomoea batatas* Lam cv White star, resulting in up to 116 embryos per 200mg of inoculum after 21 days of culture. The environment of bioreactor culture differs from that of shaker flask; preceding successful implementation of the bioreactor for large scale somatic embryo production of *Ipomoea batatas*, the shaker flask environment must be quantified and applied to the bioreactor system. Ethylene, CO_2 and O_2 levels in the headspace of shaker flask cultures were monitored (using gas chromatography) throughout the 21 day period of embryo formation. Initially the gas composition was that of room air; 0.01ppm ethylene, 0.03% CO_2 and 20.7% O_2, which changed to 0.67, 1.39 and 19.2 respectively, after the first 24 hours. By the seventh day of culture, globular and heart embryos were evident; ethylene and CO_2 increased to 3ppm and 9%, and O_2 decreased to less than 15%. Torpedo and cotyledonary development occurred after the CO_2 concentration exceeded O_2 (day 10). Mature embryos were evident by day 14 when the O_2 had been depleted to less than 10%, and CO_2 and ethylene had increased to more than 15% and 5ppm. The positive correlation of CO_2 and ethylene over time, and the negative correlation of O_2 and culture age were significant.

P-1054 Some biochemical aspects of sodium chloride salinity tolerance in Rice. V.A. Chauhan, P.C.Josekutty, L.Prakash and G.Prathapasenan, Dept. of Botany, Plant Cell & Physiology Lab., The M.S.University of Baroda, India 390 002.

A comparative study of the response of salt tolerant (CSR-1) and salt susceptible (P-106) variety of rice was carried out to understand the cellular mechanism of salt tolerance in rice. Salt adapted calli of these varieties were generated by culturing it on L.D.50 concentration of sodium chloride (Nacl). Analysis of these tissues for their levels of Na^+, K^+ and Cl^- revealed 4.9 and 3.7 fold accumulation of Na^+ and Cl^- ions respectively by P-106 as compared to that of the control. However, tissues of CSR-1 showed only 2.7 and 2.4 fold increase in the content of Na^+ and Cl^- ions respectively as compared to that of control. Contents of free sterols, steryl esters and steryl glycosides were 2.3, 3.0 and 2.1 fold respectively more than that of control in the case of CSR-1, while P-106 showed much less amount of sterols compared to CSR-1 under normal and salinised conditions. Diamine putrescine and polyamines spermidine were present in significantly high amounts in salt adapted calli. Likewise proline content of the salt adapted CSR-1 calli was much more that of salt adapted P-106 calli. These findings suggest that the ability of CSR-1 tissues to maintain a low level of Na^+ and Cl^- ions and accumulate high concentration of K^+, organic osmoticum (proline), membrane component (sterols) as well as high levels of polyamines may be rendering them more salt tolerant than those of the P-106 variety.

P-1055 Effects of Polyamines and Their Inhibitors on Organogenesis in *Euphorbia esula* L. D.G. DAVIS and P.A. Olson. USDA, Agricultural Research Service, Biosciences Research Laboratory, Fargo, North Dakota 58105, USA

The effects of the diamine putrescine (Put), the polyamines spermidine (Spd), spermine (Spm) and agmatine (Agm) on organogenesis were determined in aseptic, isolated hypocotyl and root segments of the perennial weed, leafy spurge (*Euphorbia esula* L.). Putrescine decreased root and shoot formation only if dilute nutrient media were used. Spm and Spd were inhibitory at 0.5 to 1 mM, even at full strength nutrient medium. Specific suicide inhibitors of arginine decarboxylase (ADC) and ornithine decarboxylase (ODC), both involved in the biosynthetic pathways leading to Put formation, inhibited root and shoot formation. This inhibition was partially reversed by the addition of Put. Agmatine, the intermediate between arginine and Put, did not reverse the inhibition induced by the ADC inhibitor, difluoromethylarginine. Radiolabeled Put and difluoromethylornithine (an ODC inhibitor) were taken up linearly by hypocotyl segments for up to seven days: Spd and Spm were taken up maximally within one day.

P-1056 Callus Culture and Regeneration of Sea Lettuce (Ulva lactuca L.). J.L. GALLAGHER and S. McElwee. Halophyte Biology Laboratory, College of Marine Studies, University of Delaware, Lewes, DE 19958.

Thalli of Ulva lactuca L. were collected from intertidal rocks along the Lewes-Rehoboth canal and washed in running seawater using a soft paint brush to remove epiphytes. Small pieces of thallus (1 cm^2) were incubated in 10 ml of an enriched seawater media on an orbital shaker at room temperature and a photoperiod of 15:9 (L/D) hours (83-155 μ E $m^{-2}s^{-1}$). After three months the tissue was transferred to an enriched seawater media supplemented with IAA, 2,4-D, and agar and kept in a growth chamber with a 16:8 photoperiod (93-155 μ E m^{-2} s^{-1}) and a temperature of 20°C. Some of the callus turned white and was transferred to a medium with sucrose and incubated in the dark. After two months of growth the calli were moved to standard tubes and wave tubes with several hormone concentrations. Wave tubes have agar slants with the callus at the high part of the slant. A layer of enriched seawater is placed on the slant and when shaken on the orbital shaker the wave action simulates that experienced by the plants on the rocks. Plantlets regenerated on both half-strength hormone free media. Thalli have remained small, but have developed gross anatomy similar to the field grown plants although initially they resembled the pin cushion form reported by Provasoli in axenic culture. Microscopic examination of fixed and stained sections of the tissue revealed the walls of the tubular thallus were one cell thick structures in the pin cushion form.

P-1057 Prolonged Storage of Grape Germplasm In Vitro. Influence of Low Temperature and Reduced Ammonium Nitrate Concentration.
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Vitroplantlets of wild grape species Vitis candicans Mustang., was conserved In Vitro at 10 C in the dark for 12 months, without losing their capacity to regenerate when subcultured under standard conditions. Apical and nodal explants after initial culture in Woody Plant Medium (WPM) supplemented with 0.5 uM IBA, after normal growth for 2 months at 25 C and 16/8h photoperiod, were subcultured to WPM with 100, 50, 25 and 0 percent ammonium nitrate concentrations. After growth under standard culture conditions for 4 weeks, the vitroplants were kept at 25 C and 10 C in the dark. After recording monthly observations for plant health and mortality, survival was estimated by subculturing the vitroplants after 6 and 12 months in WPM supplemented with 0.5 uM IBA. The results obtained from these experiments will be discussed in relation to the possibility of establishing an In Vitro Active Gene Bank for V. candicans species.

P-1058 Field Evaluation of Herbicide-Tolerant Hybrid Poplar Somaclones.
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Field evaluation of in vitro selected, herbicide-tolerant hybrid poplars is necessary to determine productivity traits of modified clones. In initial tests (Year 1), two glyphosate-tolerant and four sulfometuron methyl-tolerant somaclones were challenged with herbicide in replicated field trials in comparison tests to their unmodified, parent clones. Glyphosate-tolerant trees were treated with 10.5, 15.75, and 21.0 mmol/l Roundup™; sulfometuron methyl-tolerant trees with 0.69, 1.37, and 2.06 mmol/l Oust™. Stem and foliar symptoms, and tree height were recorded weekly; branch number and length, and trunk diameter were determined at the end of the growing season. Unmodified parent clones had greater mean height increase than herbicide-tolerant clones in unchallenged plots. But, with herbicide treatment, there was no significant difference in tree height. All herbicide-tolerant somaclones had increased branch number compared to parent clones. Glyphosate-tolerant somaclones achieved the greatest field tolerance. Most notably, NC-13288 survived treatment at 21.0 mmol/l while the parent clone was killed at 10.5 mmol/l. The best sulfometuron methyl-tolerant somaclone, NC-13286 had no apparent damage when treated at 1.37 mmol/l, but the parent clone, NC-11390 was killed at 0.69 mmol/l. Furthermore, all commercially important, herbicide-tolerant somaclones lacked herbicide-induced foliar damage, but has similar terminal growth damage that was observed on parent clones.

P-1059 A Model for Study disease resistance in Phaseolus vulgaris.
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Pseudomonas syringae is a pathogenic bacteria that cause halo blight in bean plants. This organism synthesizes a pathogenic toxin (Tab-toxin) which inhibits the enzyme Glutamine Synthetase. Methionine sulfoxomine (MSO) is an amino acid analogue that also inhibits the enzyme Glutamine Synthetase resulting in a decrease of glutamine and glutamate. In the current study, MSO was added to Murashige and Skoog (1962) media to simulate the pathogenic condition. Leaves were surface sterilized and explants were placed on Murashige and Skoog's media supplemented with 1 mg/l 2,4-Dichloropheny- oxyacetic acid and 0.5 mg/l Benzyladenine (standard media) for approximately 3 weeks. To increase the tolerance level, 1 gram of callus was transfered to standard media containing different concentrations of MSO: 0.25, 0.50 and 1.0 and 2.0 micromolar (uM). Callus tissue remained on each concentration for approximately 3 weeks. Callus tissue not previously exposed to MSO was also placed on standard media containing 2 uM MSO. The pre-exposed callus tissue showed a 33% increase in weight over non-exposed callus.

P-1060 *Tissue Culture Studies in Certain Mutants of Sunflower.*
T.PADMAVATHI, B.Pratibha Devi and V.Kiranmai, Department of Botany, Osmania University, Hyderabad-500007, India.

Various explants of some mutants of sunflower were tested for their callusing ability. The differences in callusing ability for various seedling explants, and the effects of certain hormones in callus initiation were studied in some mutants. Among the different explants studied, hypocotyl explants showed highest frequency followed by epicotyl, root, transition zone, stem and leaf in that order. Correlation studies between control and mutants have revealed that the controls have better callusing ability. Among the hormones tested, MS medium supplemented with NAA + Kinetin was more effective than when supplemented with IAA + Kinetin or 2,4D + Kinetin. Regeneration studies for the different calli are in progress.

P-1061 Tissue culture and Morphogenetic studies in Clusterbean (Cyamopsis tetragonoloba (L.) Taub.) **CH.AYODHYA RAMULU**, Research Student, Department of Botany, Kakatiya University, Warangal-506 009, A.P. INDIA.

The technology of isolated cell cultures on defined medium and their induction to differentiated into complete plants has resulted a wide range of applications in plant biotechnology. Plant tissue culture broadly refers to the growth and development of plant segments, tissue and various explants in aseptic environment. The various seedling explants of cluster bean grown for 3 days i.e., cotyledons, hypocotyl, epicotyl, root apex, shoot base and immature leaf cultured on modified B_5 medium supplemented with BAP, IAA, 2,4-D, and kinetin. Shoot base with 2.5mg/L 2,4-D and 1.75 mg/L Indole acetic acid in combination with other natural additives like CW (coconut water) was induced very efficient in callussination. The B_5 medium with BAP and kinetin combinations has directly differentiated into shoot buds from hypocotyland cotyledonary explants without intervention of callus formation. From all the types of calli derived from epicotyl, hypocotyl and cotyledonswere differentiated to rhizogensis while B_5 medium incorporated with 1.5 mg/L NAA and 2.5 mg/L kinetin. The direct rooting was also achieved successfully on transfer of explants to the same medium with B_5+2.5 mg/L IAA after some growth period.

P-1062 Morphogensis in Tissue Cultures of Various Explants of Cluster Bean (Cyamopsis tetragonoloba (L.) Taub.) **Ch.AYODHYA RAMULU** and Digamber Rao, (ARC,DR), Department of Botany, Kakatiya University, Warangal-506009, INDIA.

The technology of isolated cell cultures on defined medium and their induction to differentiated into complete plants has resulted a wide range of application of tissue culture of current interest. Plant tissue culture broadly refers to the growth and development of plant segments, tissue and various explants in aseptic environment. The various seedling explants of cluster bean grown for 3 days i.e., cotyledons, hypocotyl, epicotyl, root apex, shoot base and immature leaf cultured on modified B_5 medium supplemented with BAP, IAA, 2,4-D, and kinetin. Shoot base with 2.5 mg/L 2,4-D and 1.75 mg/L Indole acetic acid in combination with other natural additives like CW (coconut water)was induced very efficient in callussination. The B_5 medium with BAP and kinetin combinations has directly differentiated into shoot buds from hypocotyl and cotyledonary explants without intervention of callus formation. From all the types of calli derived from epicotyl, hypocotyl and cotyledons were differentiated to rhizogensis while B_5 medium incorporated with 1.5 mg/L NAA and 2.5 mg/L kinetin. The direct rooting was also achieved successfully on transfer of explants to the same medium with B_5+2.5 mg/L IAA after some growth.

P-1063 Development of Embryogenic Tissue Culture and Subsequent Plant Regeneration From *Paspalum notatum* Fluegge 'Tifton 9'. R.A. Wheeler, R.G. SHATTERS,JR. and S.H. West. USDA, ARS, Plant Stress and Protection Research Unit, Gainesville, FL 32611.

Paspalum notatum Fluegge (Pensacola bahiagrasss) is a major tropical forage crop in the southeastern United States. Bahiagrass has excellent drought tolerance and satisfactory forage quality. Productivity, however, is limited to only about six months out of a year. This limitation is due to environmental stresses including draught, suboptimal temperature and limited irradiance during the winter months. As part of a research project to analyze the molecular response of this grass to environmental stress, we have successfully developed an embryogenic tissue culture line of a highly productive bahiagrass cultivar Tifton 9. This line was developed from cross-section explants near the meristematic tissue at the base of the leaves. Initial nonembryogenic tissue gave rise to embryogenic callus after approximately 3 weeks of explant culture. Embryogenic tissue was continuously cultured for six months and maintained its embryogenic nature. Embryos germinated upon placement onto media without plant growth regulators allowing isolation of as many as ten to twenty regenerated plants per 0.5 cm diameter callus sections. Plants could be potted in the greenhouse after 3 weeks on non-growth regulator medium.

P-1064 In vitro adventitious root production in Artemesia annua L.

S. VENKETESWARAN, H. Nguyen, T. Doung, H. Samartzidou,N. Kletzly, Department of Biology, University of Houston, Houston, TX. 77204 and Peter K. Chen, Department of Biology, Georgetown University, Washington, D.C. 20004.

Seeds of Artemesia annua L. were germinated aseptically on a modified Murashige & Skoog medium (MS). 1-2 mm. segments of hypocotyls or young stems were transferred to MS medium supplemented with different plant growth hormone combinations and concentrations. Callus cultures initiated and grew well on MS + 1 mg/l each of kinetin and 2,4-dichlorophenoxyacetic acid (2,4-D). Liquid suspension cultures were established and maintained on a mechanial shaker (1 rpm). Large amounts of cell clumps and cell suspension could be obtained when grown in large containers. Intense adventitious root production was produced on 1/2 MS medium + 3 mg/l of α-naphthalene acetic acid (NAA) on both soild and liquid cultures. These roots resembled in growth and characteristics of "hairy root" lines generally produced by transformation with *Agrobacterium rhizogenes*. The potential use of adventitious root production in Artemesia anuua for secondary metabolite production, e.g. 'Artemesinin' appears very promising. (Supported in part by Universityof Houston Coastal Center, Houston, TX).

P-1065 Somatic Embryogenesis in Helianthus Porteri (A. Gray) Heiser. W.F. Yates. Department of Biological Sciences, Butler University, Indianapolis, IN 46208, USA

Although many protocols have been devised for in vitro propagation of cultivated sunflowers, little attention has been given to propagation of wild species which might provide new genes or serve as conduits for introducing genetic material from other genera. Helianthus Porteri, although of no economic value, displays an unusual propensity for hybridization with other sunflowers and, in at least one instance, with another genus. Many of these hybrids are highly sterile and require some mode of propagation before further hybridization. Efforts to achieve somatic embryogenesis in H. Porteri were initiated with the hope that the same protocol might be successful with any hybrid progeny obtained. Seedlings of H. Porteri were germinated aseptically and explants taken from the basal regions of cotyledons one cm in length. These were placed on a modified MS medium containing 4% sucrose, 1 mg/l NAA, 0.2 mg/l BAP, and 300 mg/l l-glutamine. In 4 weeks, green callus was formed and somatic embryos were observed on this callus. Embryogenic callus was transferred to a development medium similar to the initial medium but with 1 mg/l IBA as the only growth substance. Shoot development and rhizogenesis occurred with ca 25% of the initial embryos showing further growth.

P-1066 Tissue Culture and Regeneration in the Halophytic Grass *Spartina alterniflora* Loisel. XIANGGAN LI, D.M. Seliskar, J. Moga and J.L. Gallagher, Halophyte Biology Laboratory, College of Marine Studies, University of Delaware, Lewes, DE 19958

Smooth cordgrass, *Spartina alterniflora* Loisel.(Poaceae), is a perennial grass dominating high salinity marshes of the Atlantic and Gulf coastline. It grows very well in seawater with higher biomass production. Callus was initiated from seedling shoot meristem on ADM medium (MS salts + 1.0 mg /l IAA and 2,4-D + 5% coconut water). BND (MS salts + 0.5, 1.0, and 0.5 mg/l BAP, NAA and 2,4-D± 5% coconut water) was better than ADM and MSD (MS salts + 2 mg/l 2,4-D) for maintenance of calli. More calli were induced on medium with high sucrose (13%) than low sucrose (3%). Shoots were formed from 81% and 92% of the cultures grown on MS medium containing 1.0 mg/l BAP or 3.0 mg/l BAP + 0.2 mg/l IAA respectively. No difference in shoot formation was found between cultures on MS + 1.0 mg/l BAP and half strength of MS + BAP 1.0 mg/l. Root formation from calli was observed on MS medium + BAP and NAA. Since this species is very salt-tolerant and ecologically important in salt marsh replenishment, this callus culture should be useful for studying salt tolerance mechanisms and in selecting somaclonal variants.

P-1067 Induction of Differentiation and Regeneration of Plants, Establishment of Fine Cell Suspension Cultures and Protoplast Isolation in Indica Rice Varieties. M.S. KHINDA, S.S. Gosal, J.S.Sandhu and H.S. Dhaliwal. Plant Biotechnology Centre, Punjab Agricultural University, Ludhiana-141004, India

Callus cultures were established from mature seeds of 7 indica rices viz. PR103, PR106, PR109, IR8, basmati 370, Pusa basmati 1 and Vaigai. Best callus induction was obtained on MS + 2,4-D (2 mg/l) + Kin (0.5 mg/l). Nodular sectors appeared on the calli during their maintenance. Such calli exhibited the development of green islets resembling shoot buds on regeneration mediums viz. MS + BAP (1.0 mg/l) + Kin (1.0 mg/l) + NAA (0.1 mg/l) and MS + BAP (2.0 mg/l) + NAA (0.1 mg/l). Normal shoots and their subsequent rooting was obtained in three varieties. Fine cell suspension was developed in two varieties viz. Pusa basmati-1 and Vaigai in liquid medium. Addition of Tyrosine (50 mg/l), tryptophan (50 mg/l) and alanine (50 mg/l) improved the growth of cell suspension cultures. The cells harvested from suspension cultures incubated in cellulase R-10 (1.5%) and macrozyme R-10 (0.5%) for 8-10 hours, gave better yields of protoplats as compared to actively growing calli.

P-1068 Somaclonal Variation - A Source of Genetic Diversity in safflower, Carthamus tinctorius L. P. SEETA and S.Y. Anwar, Department of Genetics, Osmania University, Hyderabad - 500 007, India.

Regenerants (somaclones) intiated from cotyledon explants of genotype Mangira, displayed genetic (heritable) changes in following regenerations. Some of the variants observed in the Sc_1 generation will be described. These include variants for plant height, leaf shape, plant type, capitulum, flower colour and seed shape. Variants which exhibited precocious flowering and sterility were also recovered. Variation for quantitative characters was recovered for characters like days to flowering and maturity, number of primary and secondary branches per plant, number of capitula per plant and seed yield per plant. The somaclonal variation recovered in the Sc_1 generation, was expressed in the selfed progeny of somaclones (Sc_2 generation), suggesting that these variants were true breeding somaclones. The extensive variation both for qualitative and quantitative characters observed in regenerants suggests that tissue culture induced variation can be recovered in the regenerants which may be successfully utilized in the subsequent breeding programmes.

P-1069 Anther Culture of Indica Rice Varieties. J.S. SANDHU, M.S. Gill, S.S. Gosal, M.S.Khinda and H.S. Dhaliwal. Plant Biotechnology Centre, Department of Plant Breeding, Punjab Agricultural University, Ludhiana-141004, India

Anthers containing pollen at uninucleate stage from three commercial indica rice varieties viz. Jaya, IR54, Vaigai were cultured on seven media compositions based on N_6 salts. In general, the cultured anthers exhibited browning in all the cases after 7-10 days of culturing. The anthers started callusing after 45 days of incubation at 25±1°C and continued to callus upto 100 days. The callusing frequency varied with the composition of medium, genotype of donor and the intensity of illumination.Per cent callusing ranged from 1.75%(Jaya) to 3.5%(IR54). Best callus induction was observed on N_6 medium supplemented with 2,4-D (1.75 mg/l) + Kin (0.5 mg/l) + Sucrose (3%). Anther derived calli were creamish white in colour and friable in nature. The calli subcultured on N_6 medium supplemented with BAP (0.5 mg/l) + Sucrose (4.5%) exhited the development of green iselets and subsequent plant regeneration ranged from 15% (Jaya) to 23% (IR54).

P-1070 Somatic Embryogenesis and Plant Regeneration from Callus Cultures of Finger millet. A.M.RAO. Tissue Culture Lab, Department of Botany, Kakatiya University, Warangal 506009 INDIA.

Genotypic difference for callus induction, percent frequency in plant let regeneration and mean number of plants formed per 200 mg of callus were observed in finger millet (Eleusine coracana). Both the cytokinin BAP (2.0 mg/l) and kinetin (2.0 mg/l) proved to be effective in induction of somatic embryogenesis. Sucrose as a carbon source has a profound influence on shoot organogenesis. High frequency and plant regeneration was observed from 165 to 180 days of static cultures and after a long period while maintaining the culture on similar cultural conditons the regeneration capacity was totally ceased.

P-1071 High Frequency of Plant Regeneration From Callus Cultures of Paspalum scrobiculatum. A.M. RAO. Tissue Culture Lab, Department of Botany, Kakatiya University, Warangal 506 009, INDIA.

Plant tissue culture has many applied values in crop improvement programmes includes the change in plant archetecture to morpogenesis. Kodo millet (Paspalum) is unknown in a wild state as true millet in the history. The dehusked grain is considerably nutritions and eaten whole boiled or cooked and major supplement to rice. Genotypic difference for callus induction, percentage in frequency.of plant let regeneration and mean number of plants formed per 200 mg of callus were observed in kodo millet on M.S medium. Among various phytohormones incorporated in the M.S medium BAP (2.0 mg/l).was found to be suitable for high frequency of plant let regeneration. Manipulation of sucrose has.also a profound to be influence on shoot organogenesis. High frequency plant regeneration was observed upto 160 to 180 days while subculturing. Regeneration capacity of callus cultures was lost during the period of 200 days of maintainence as static cultures.

P-1072 Somatic Embryogenesis in Hybrid *Liriodendron*. S.A. MERKLE[1], M.T. Hoey[1], B.A. Watson-Pauley[1] and S.E. Schlarbaum[2]. [1]School of Forest Resources, University of Georgia, Athens, GA 30602 and [2]Department of Forestry, Wildlife and Fisheries, University of Tennessee, Knoxville, TN 37901.

Propagation of rare hybrids between the North American yellow-poplar (*Liriodendron tulipifera*) and the Chinese tuliptree (*Liriodendron chinense*) could be greatly accelerated with the availability of a high frequency embryogenic system. Chinese tuliptree flowers were collected from a tree growing at an arboretum in Virginia and the anthers were used for controlled pollinations of 4 yellow-poplar mother trees. Aggregates of samaras resulting from the pollinations were harvested 8 weeks postpollination. Following surface sterilization, samaras were dissected and embryos and endosperm were cultured together on a semisolid induction medium containing 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 0.25 mg/l benzylaminopurine (BA), 40 g/l sucrose and 1 g/l casein hydrolysate (CH) in the dark at 25° C. Following 2-3 months on induction medium, an average of 12 percent of the explants produced either somatic embryos or proembryogenic masses (PEMs). Compared to pure yellow-poplar embryogenic cultures, putative hybrid cultures grew slowly and tended not to maintain growth as PEMs even while exposed to 2 mg/l 2,4-D, instead proliferating via repetitive embryogenesis as globular-stage embryos. Four to 6 weeks following transfer of globular embryos to basal medium, mature embryos were produced from 4 of the putative hybrid lines. Mature embryos transferred to basal medium lacking CH and cultured in the light at 25° C greened and germinated. Plantlets survived transfer to soil mix and acclimatization to greenhouse conditions. Hybrid genotypes of the cultures, embryos and plantlets are being verified using DNA markers from both parental species.

P-1073 Multiple tillering through Secondary Embryogenesis in anther culture of indica rice. N.LENKA and G.M.Reddy. Department of Genetics, Osmania University, Hyderabad - 7 India.

Cereals especially indica rice is mostly genotype dependant in terms of anther culturability, where efficient green plant regeneration and high frequency of albinos are the bottle neck. Somatic embryogenesis plays a critical role in the field of micropropagation as well as direct gene transfer thus avoiding the risk of plant regeneration to some extent. A system has been developed to obtain large number of plantlets through multiple tillering of the rgenerating shoot buds from anther callus of indica rice; cv.Ptb-33. The auxin, 2,4-D instead of NAA - 2 mg/l each in combination with 0.5mg/l Kinetin and 6% sucrose in the callus induction medium influenced mostly in obtaining the embryogenic callus which inturn modulated plant regeneration. Around 20-30 shoot buds developed at the base of individual shoot regenerant on MS regeneration medium based on the type and concentration of hormones used. Each shoot bud (3-5mm length) subsequently exhibited multiple budding through secondary embryogenesis upon transfer to fresh MS medium. 2mg/l BAP along with 1mg/l NAA, 0.6mg/l Kinetin and 6% sucrose played a key role in this respect. Around 400 plants have been successfully regenerated and transferred to net house. Some of the regenerants have shown certain superior agronomic traits like early maturity and higher yield potential when compared to control. The present finding can be of great help in the field of gene manipulation and artificial seed production.

P-1074 Hormone-Mediated Regulation of Fatty Acids Production in Calli of Euphorbia characias L.

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Calli of E. characias maintained under different auxin and citokinin supplementations revealed significant differences in the amounts of fatty acids produced. 2,4-Dichlorophenoxiacetic acid (2,4-D) at concentrations lower than $1x10^{-6}$M was more effective on the promotion of fatty acids biosynthesis than indole-3-butyric acid (IBA), indole-3-acetic acid (IAA) and alpha naphthalene acetic acid (NAA) tested at the same concentrations. However, contrarily to the other auxins, the increase of the 2,4-D concentration above 1×10^{-6} M promoted a decrease in the production. NAA was the auxin less effective for fatty acids production. Calli maintained with IBA and IAA at concentrations above 1×10^{-6} M promoted a production of fatty acids higher than those maintained with 2,4-D or NAA at the same concentrations.

The increase of the concentrations of benzyladenine (BA) and kinetin (KIN) above $1x10^{-6}$M showed also an inhibitory effect on the fatty acids acumulation while zeatin (ZEA) had an increasing effect.

P-1075 Long Term Preservation of Embryogenic Cell Cultures of Birch (Betula pendula Roth). L.M. MANNONEN and W.A. Monger. VTT Biotechnical laboratory, SF-02150 Espoo, Finland.

Embryogenic potential of plant cell cultures is easily lost during prolonged culture in undifferentiated state. To avoid this a long-term preservation method is needed. Also considerable savings in materials, time and effort is achieved through a prolonged sub-culture interval.

Preservation may be done by slowing down the metabolism or preferably by halting the whole metabolism. In this study embryogenic cell cultures of birch were preserved under mineral oil at 24 °C for up to 18 months and in liquid nitrogen for 6 months. Embryogenic potential was recorded before and after preservation on solidified embryo production medium by counting the embryos. Preservation under mineral oil was done as described by Mannonen et al. (1990). Cryopreservation was done with a modification of the method presented by Kartha et al. (1987). Embryogenic callus cultures were suspended in liquid growth medium. After 24h culture the suspension was sieved through a 1000 μm screen and precultured at 0,2M sorbitol for 24h and at 0,4M sorbitol for another 24h. For cryoprotection 5% DMSO was added. Freezing was done at -0,5°C/min to -35 °C whereafter the ampoules were plunged in LN.

The birch callus survived even 18 months mineral oil preservation and growth commenced rapidly right after transfer to fresh medium. However, the embryogenic capacity was greatly reduced already after 3 months storage and after 1 year hardly any embryos could be induced from the regenerated cell mass. On the other hand, cryogenic storage proved to be an excellent method for preserving the embryogenic properties of all tested cell lines. Cryopreservation seemed even to enrich the embryogenic proportion of the cells. Furthermore cryogenic storage has no limitations on preservation time.

Ref. Kartha KK et al. (1987) Plant Cell Rep. 6, 480-483
Mannonen L et al. (1990) Plant Cell rep. 9, 173-177

P-1076 Selection of Glyphosate-Tolerant Tobacco Variants from Callus Cultures. M.NEDKOVSKA Institute of Genetics, BAS, Sofia 1113, BULGARIA.

Tissue cultures methods in addition to conventional breeding programs have been employed in the search for herbicide tolerance. The development of herbicide-tolerant plants has significant agronomic implications and could enhance benefits of herbicides. The objective of this study was the selection of herbicide-tolerant variants of important Bulgarian tobacco cultivars from callus cultures and expression of this tolerance in regenerated plants. The herbicide used in this investigation was Glyphosate-nonselective broad spectrum herbicide. It is a potent inhibitor of the shikimate pathway enzyme 5-enol-shikimate-3-phosphate (EPSP) synthaze-a key enzyme in the biosynthesis of aromatic acids in bacteria and plants. We used stepwise selection from 60-300mg/l Glyphosate in selective medium. The studies showed that the high dose of the herbicide (300mg/l) is a concentration which can be used as a basis for selection of Glyphosate tolerant callus clones. Regeneration in callus cultures grown on high doses of Glyphosate (80,100mg/l) in the medium was observed, when the concentration was reduced to 40mg/l Glyphosate. Attempts to regenerate fertile plants from tolerant callus lines were successful. Also we are trying to limit the adaptation time for avoiding the increase of genetic damages in the callus lines. Experiments on R-plant tolerance sexual transmission are in progress.

P-1077

Pollen Cultures of Pepper(Capsicum annuum L.)

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Pollen cultures are an effective and precise method in obtaining haploids. There are no data for their application in pepper. This study represents our preliminary results in elaborating of a suitable technique and nutrient medium for pepper.
Plants of three varieties (cv.Sivriya, Yolo Wonder and Pazardzishka kapiya) were used as donors. Modified Durand et al.(1973) liquid medium for protoplasts, enriched with 1 mg/l NAA, 1 mg/l BAP, 1 mg/l 2,4-D and 0.5 M manitol was used as a culture medium. Sterilized anthers were gently pressed with pestle into the nutrient medium, then filtered through nylon mesh with pore size of 53 μm and thrice centrifuged at 100 X g for 3 min (Lichter et al., 1982). Isolated pollen was cultured in petri dishes at a density of about 2 X 10^5 per ml, at 25°C, in dark. During the first 15-day culture period initial divisions in microspores of the three varieties were observed, with prevalance of 3-4 nuclear cells. In cv.P.kapiya rare appearance of pollen dimorphism was examined. Bigger pollen grains passed through several divisions, forming multicellular structures. Prevalance of microcalli was observed in cv.Yolo Wonder. Rare multinuclear grains and microcalli were formed in cv.Sivriya pollen cultures.

P-1078

Optimization of rice protoplast nurse culture using the filter method. J.R. AMBLER, J.W. Seay and T.C. Hall. Department of Biology, Texas A&M University, College Station, TX 77843.

For reliable growth of rice protoplasts after transformation by electroporation-mediated DNA uptake, culture with nurse cells is required. We are using the convenient filter method of nurse culture; protoplasts are cultured on a membrane filter overlaying culture (previously grown in suspension) on a plate of agarose-solidfied protoplast culture medium. In our early transformation studies, small variations in conditions for pre-growing suspension culture greatly affected plating efficiency. Thus, we have conducted experiments to optimize nurse culturing. Two of the most critical factors for success are (1) use of a nurse culture which does not acidify culture medium rapidly and (2) minimization of the osmotic shock when transferring nurse cells from 2% sucrose culture medium to 10% glucose protoplast medium. The culture to be used for nurse must be grown continuously at low density because, if the culture medium becomes too acidic during one passage, it will continue to acidify the medium excessively the next passage. Osmotic shock may be minimized by (1) addition of mannitol to the nurse culture stock medium, (2) gradual adjustment of osmotic concentration prior to plating nurse cells for protoplast culture, and/or (3) covering nurse cells with low sugar medium rather than protoplast medium. Results from studies on other variables will also be presented.

P-1079

WITHDRAWN

P-1080

Carbohydrate Metabolism during Somatic Embryogenesis in Carrot

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When somatic embryogenic callus is forming, dramatic cytochemical changes are occurred by shifted gene expression. For example, it has been emphasized the remarkable emergence of starch grain in embryogenic callus cell which might be resulted from changed metabolic activities.

In order to analyze carbohydrate metabolism, various carbohydrate contents were measured in batch cultures of *Daucus carota* which was undergoing somatic embryogenesis. In addition, major enzyme activities related with carbohydrate metabolism were assayed on the various stages. The starch content was rapidly increased during the early stages of embryogenic callus formation and increased α-amylase activity was followed. Glucose and sucrose contents were almost doubled in embryogenic callus than them of nonembryogenic callus and increased at the following stages. The transition of carbohydrate metabolic activity during development of somatic embryo seems to be significant as expected through observations of morphological changes. Distributional charateristics of starch at the each developmental stages were also observed. And detrimental effects on growth and development of somatic embryogenesis are discussed.

P-1081 Daucus carota: Characterization of Cell Lines and Plants Resistant to Chlorsulfuron. S. Caretto , M. C. Giardina, C. Nicolodi and D. MARIOTTI. IREV-CNR, Via Salaria km. 29,300, 00016 Monterotondo Scalo (RM), Italy.

The herbicide chlorsulfuron (CS), which acts by inhibiting the branched-chain aminoacid biosynthesis through the inhibition of acetolactate synthase (ALS), has been used to select resistant carrot cell lines. Increasing concentrations (up to 10^{-7}) were used and three callus lines have been selected resistant to 10^{-8} (SC10), $5*10^{-8}$ (SC50) and 10^{-7} (SC100), respectively. In order to verify the stability of the observed resistance callus-derived suspension cultures have been first mantained in herbicide-free medium and thus retested for resistance. The CS inhibition profiles of ALS isolated from the selected lines showed that SC50 and SC100 were definitely less sensitive to the herbicide than controls, while SC10 did not show any significant difference. Moreover ALS activity showed a significant increase in SC50 and SC100. This could be due to an increase of the gene copy number as described for other herbicides following a stepwise selection. Southern hybridization carried out with DNA from the selected lines seems to confirm our hypothesis. Plantlets have been regenerated from SC50 cell lines and experiments are in progress to characterize the resistance at whole plant level.

P-1082

WITHDRAWN

P-1083 Shoot Differentiation and Plant Regeneration of Artocarpus heterophyllus L am. Through Callus Culture. S.K. Roy, T. Hossain and M.S. Islam. Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh.

Shoot differentiation and regeneration of plants has been acheived in callus culture of Artocarpus heterophyllus using young leaves and stem segments as explants from mature plants. The explants were cultured aseptically on Murashige and Skoog (MS) based medium. Callus was initiated from the cut ends of the explants on MS containing 2, 4-D or NAA at 1-3 mgl^{-1} within two weeks. Large amount of friable callus was obtained on media supplemented with 2, 4-D(1.0 mgl^{-1}) or NAA (3 mgl^{-1}) alone in about 5-6 weeks. But these calli were not suitable for shoot induction. Hence, the combinations were changed and the MS medium subsequently supplemented with NAA + BA and CM and CH separately. Thus the MS medium with 3 mgl^{-1} NAA (or 1 mgl^{-1} 2, 4-D) + 1 mgl^{-1} BA + 10% (v/v) CM was found to produce morphogenetic calli, whereas addition of CH was not suitable. The passage time of the calli was four weeks. These morphogenetic calli when cultured on MS medium with 2.5 mgl^{-1} BA + 0.5 mgl^{-1} NAA produced maximum number of shoot buds. After four weeks these shoot buds along with calli were transferred to fresh MS medium containing the same hormonal combination, where the old shoot buds were elongated and many new buds were also emerged. The shoots were then individually rescued and cultured, for axial growth, in MS + 0.05 mgl^{-1} BA medium. For rooting they were then implanted onto the medium containing half-strength MS salts + 0.2 mgl^{-1} each of IAA and IBA. Rooted plantlets were planted on soil.

P-1084 The Effect of Sugars and Growth Regulators on Embryoid Formation and Plant Regeneration from Barley Anther Culture. Q.H. CAI and I. SZAREJKO. Institute of Nuclear Agricultural Sciences, Zhejiang Agricultural University, Hangzhou 310029, P.R. of China

The influence of carbon source and growth regulator composition in induction medium on anther culture response was investigated using spring barley genotypes. Anthers were cultured on BAC3, Ficoll-containing induction medium, supplemented with one of the following carbohydrates: sucrose, maltose, cellobiose and melibiose (6% w/v). The use of either maltose or cellobiose resulted in a significantly higher anther response, calli and/or embryoid production and green plant regeneration compared to the incubation of anthers on a medium containing sucrose while melibiose drastically reduced the efficiency of anther culture. As an average for three genotypes tested, the frequency of green plants per 100 anthers plated was 9- to 22-fold higher on medium supplemented with sucrose or cellobiose than on medium containing melibiose as a sole carbohydrate. Among the growth regulators tested, the combination of auxin NAA (2mg/l) and cytokinin BAP (1mg/l) performed much better than the employment of auxin 2,4-D combined either with zeatin riboside or BAP as cytokinins. The benefitial effect of medium supplemented with NAA and BAP was connected with better embryoid formation compared to the other growth regulator combinations tested.

P-1085 **Isolation of High Yielding Line Through Tissue Culture in Carthamus Tinctorious L. (Safflower)**

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Carthamus tinctorious L. var HUS selected for the the work. Leaves, cotyledons, hypocotyl and roots were used as explants. 0.2% mercuric chloride solution used as sterilizing agent. The explants inoculated onto the different media including MS with different hormonal concentrations. Out of these MS medium with 2,4-D (2 mg/l)+IAA (0.05 mg/l) found most suitable for callus initiation from all explants taken. Callus from different explants transferred to shooting media of MS medium. Multiple shooting obtained in BAP (4.0 mg/l)+NAA (0.5 mg/l) concentrations. Roots produced to these cultures by the action of GA 3 (1.0 mg/l) + NAA (1.0 mg/l) concentration. The regenerated plants transferred to the field and their morphological, anatomical and physiological variations observed. 60% of the field transferred plants survived upto the harvesting stage. The plants obtained from different explants showed different variation in their morphological and physiological characters. Plants obtained from cotyledon explants showed more frequency of variation which includes a set of 6 plantlets which at the stage of harvesting produced 30% more yield than control plants. The seeds from these six plants collected and multiplied for two generations to test the stability of genotypic character. Both generations showed increase in the yield.

P-1086

WITHDRAWN

P-1087 Somatic Embryogenesis In Vigna radiata (L) Wilczek. A.MADHU MOHAN RAO and S.A.Farooq. Tissue culture laboratory, Department of Botany, Osmania University, Hyderabad - 500007, INDIA.

High frequency embryoid induction has been obtained from seedling explants (hypocotyl and cotyledons) of Vigna radiata (L) Wilczek. Explants were cultured in vitro on Murashige and Skoog and B_5 media supplemented with one or more auxin and cytokinin. The growth regulators responded variably in callus induction. The callus was friable and white with green and compact patches. This compact green callus was sub-cultured and multiplied on MS medium with lower levels of 2,4-D. The callus was sub-cultured and multiplied on both media containing various combinations or different growth regulators. The best response for embryoid initiation was noted on a combination of BA and 2,4-D in both explants. Histology and differentiation of embroids and callus formation has been worked out in detail.

P-1088

WITHDRAWN

P-1089 Tissue culture and Biochemical Studies of Alternaria Resistance in Safflower (Carthamus tinctorius L) M.A.KHADEER and S.Y. Anwar, Department of Genetics, Osmania University, Hyderabad - 7, India.

Safflower, an important minor oilseed crop of India loses about 10-12% of seed yield due to Alternaria blight. Attempts to develop the disease resistant line by conventional methods have been futile. Hence, the alternate; non-conventional method of In Vitro selection was initiated with a twin objective of a) Selection of Alternaria resistance and b) studying the biochemical changes associated with disease development.

Eleven germplasm accessions/varieties were selected for the present study. Cotyledonary explants of both resistant and susceptible cultivars were grown on culture filtrate supplemented MS medium (2,4-D 2mg/1 + 15% CM). 500mg of leaf/calli collected every 24hrs. for 7 days was used to study the effect of culture filterate on peroxidase activity & protein content in one month old calli and one week old seedling.

Decrease in protein content and enzyme activity were seen in stressed calli and seedlings. Attempts to regenerate plants are on the way. Thus the present In Vitro selection method would be a potent genetic tool in supplementing the traditional breeding programs in safflower improvement.

P-1090

WITHDRAWN

P-1091

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P-1092

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P-1093

WITHDRAWN

P-1094 In vitro Shoot Proliferation of Astrophytum capricorne. M.C. Ojeda[1], E. CARDENAS[2], T.E. Torres[3] and J. Verduzco[4]. (1, 2) Fac. de Agronomía U.A.N.L. Apdo. Postal 358. San Nicolás de los Gza., N.L. 66400. (3, 4) Fac. de Ciencias Biológicas U.A.N.L. Apdo. Postal 2790. San Nicolás de los Gza., N.L. México.

Astrophytum capricorne is a cactus species included on the preliminary list of rare, threatened and endangered plant species of México. Tissue Culture techniques had shown be a rapid and efficient method for mass propagation of endangered species. This work report an in vitro system for the induction and shoot proliferation of Astrophytum capricorne from meristems of aseptically germinated seedlings. Seeds pre-desinfestation involved rinsing with tap water, then surface sterilizing in a 1.0% solution of sodium hypochlorite for 10 minutes. The seeds were germinated on 0.8% of agar-agar. After six weeks the seedlings epicotyls were excised and transferred to Murashige-Skoog medium supplemented with 100 mg. L^{-1} myo-inositol, 3.0 mg.L^{-1} NAA, 3% sucrose and 0.5% gelling agent. The induction stage was obtained in this medium after two weeks on culture, where was evident surface and areole growth. Areole explants were subcultured on proliferation shoot MS media containing 0.1 mg.L^{-1} kinetin and 0.2 mg.L^{-1} BA or 5.0 mg.L^{-1} BA and 0.1 mg.L^{-1} NAA. Maxium axillary shooting were obtained with both citokinins media from areoles, which results in advantage because plants regenerated could be considered more genetically stable.

P-1095 Evaluation of Somaclonal Variation in Ornamental Plants. S. MOHAN JAIN, Department of Crop Husbandry, University of Helsinki, SF-00710, Helsinki, Finland

Somaclonal variation in Begonia x elatior and saintpaulia regenerated ornamental plants were studied. Shoots were differentiated from saintpaulia leaf disks on MS medium containing 0.1 mg/l BAP, which were rooted on MS medium having 0.1 mg/l NAA. Over 1000 regenerated plantlets were evaluated in the greenhouse. No variation was seen in flower color and flower size. However, there were differences in flower morphology, number of petals/flower, flowering time, and number of flowers/plant. Plants flowered as early as 76 days and as late as 106 days. About 47% regenerated plants did not flower and 0.3% plants had an average of 23 flowers/plant. Flower corolla was tubular in 3-4% plants and abcissed by a gentle touch. Flowering did not take place in 180 plants. These plants had hard, thick, and in some plants, variegated leaves and their growth was slow. From Begonia leaf disks, callus was initiated on MS medium containing 0.1 mg/l NAA, 0.2 mg/l 2,4-D, and 0.1 mg/l BAP. After 4-5 weeks, Begonia callus was differentiated into shoots on B5 medium having 0.1 mg/l zeatin and 1.0 mg/l kinetin. Regenerated shoots were rooted on MS medium amended with 0.1 mg/l NAA. A total of 336 regenerated plantlets were evaluated in the green house. No variation in flower color was observed. However, 2% regenerants had an average 50 flowers/plant as compared to 25 flowers/plant in the control. In our results, flower color seems to be a stable character under tissue culture conditions. Selected plantlets were micropropagated and resulted in stable lines.

P-1096 Physiological Aspects of Vitrified Plants during Transformation to Normal Morphology. T.W. ZIMMERMAN and B.G. Cobb. Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843-2133.

A synchronized system was developed for reverting vitreous plant growth to normal by limiting the supply of medium. As the supply of water and nutrients became limiting, the shoot apex of petunia was transformed from a vitreous state to normal. Subsequent changes occurred in vitreous plants which included: reduction of the growth rate; leaves, beginning at the base and moving acropetally, lost their watersoaked appearance; increased leaf glucose, sucrose and inositol with no change in fructose concentrations. 2-D electrophoresis, run on extracted proteins from shoot apical regions, revealed unique proteins present in vitreous shoots but absent in normal shoots.

P-1097 Callus Induction and Regeneration in Leaf Blade Culture of Beta Species. P. AHMADIAN-TEHRANI and M. MESBAH.University of Tehran and S.B.S.I. Karaj, 31587 IRAN

The ability to regenerate shoots from beet callus tissue was investigated in an experiment with leaf blade culture of whole plants of eight Beta species including: sugar, table and fodder beets and two wild native species. Two sets of MS and modified B5 (PGOB) media supplemented with 1 μM BA solely or accompanied either by 155 μM ascorbic acid or 100 mg/l adenine sulfate were used. Different degrees of callus formation and proliferation between the leaf sources, and a germplasm-medium interaction was observed. Both basic media containing BA, and BA+Vit C proved to be suitable for callus induction in general. Shoot regeneration occurred in friable callus of the fodder beet on PGOB+BA+Vit C after 6 weeks at 30°C, in dark. Additional cases of shoot formation in fodder and sugar beet callus tissue and also directly on the leaf pieces of the fodder beet occurred after the transferring to light on MS media containing 1 μM BA.

P-1098 Establishment and Maintenance of Embryogenic Tissue Cultures of Pinus strobus L. K. Kaul, CRS Plant and Soil Science Research, Kentucky State University, Frankfort, KY 40601.

Attempts were made to obtain embryogenic tissue cultures using female gametophyte of Pinus strobus as explant. Influence of developmental stage and genetic make-up of the explant, and the composition of the culture medium on proliferation of embryogenic tissue were examined. Maturing female cones were collected from seven 25 to 30 years-old trees belonging to five different clones. Seeds were removed from the cones and surface sterilized. Female gametophyte was dissected out aseptically, placed on one of the three experimental media, and incubated at 21°C in darkness. The observations made suggest that (1) the potential for proliferation of embryogenic tissue varies based on the genetic make-up of the explant; (2) the potential for proliferation of embryogenic tissue exists only for a short period of time during seed development; and (3) the composition of culture medium greatly influences the proliferation of embryogenic tissue. A total of 16 embryogenic tissue lines have been isolated. Twelve of these lines are being maintained by periodic subculturing. Fully developed somatic embryos have not been obtained from any of the tissue lines.

P-1099 Micropropagation of Texas Madrone, Arbutus texana. 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 Texas Madrone, a native tree of west Texas, and successfully cultured on basal medium consisting of WPM salts, MS vitamins, $30g \cdot l^{-1}$ sucrose, 0.8% Phytagar supplemented with 2.5 $mg \cdot l^{-1}$ BA. Shoots were subcultured every 4 weeks on the same medium to obtain sufficient culture material for experiments. Basal medium was supplemented with BA (0.5, 1.0, 2.5, 5.0, and 10.0 $mg \cdot l^{-1}$) and NAA (0, 0.1, 1.0 $mg \cdot l^{-1}$) in factorial combination. Highest shoot (both axillary and adventitious) proliferation occurred on basal medium supplemented with BA only. Rooting occurred on basal medium containing NAA with either no or low concentrations of BA. Excessive callus formation occurred on medium containing high BA concentrations combined with 1.0 $mg \cdot l^{-1}$.

P-1100 Micropropagation of Sequoia sempervirens. I.W. SUL and S.S. Korban. Department of Horticulture, University of Illinois, Urbana, IL 61801.

An in vitro micropropagation system for Sequoia sempervirens using stem segments (1 cm) with 2 to 5 axillary buds was developed. The influence of the following cytokinins, thidiazuron (TDZ), 6-benzylaminopurine (BA), N-benzyl-9-(2-tetrahydrophyranyl)adenine (BPA), kinetin, 6-(r,r-dimethyallylamino)-purine (2ip), and zeatin on shoot proliferation was investigated. In addition, we investigated the combined effect of BA and adenine hemisulfate on proliferation. All treatments were conducted using Wolter and Skoog (WS) (1966) medium supplemented with Staba vitamins. Among cytokinins, zeatin induced both bud break and shoot elongation; whereas, the other cytokinins induced only axillary bud break. In addition, all stem segments cultured on WS medium containing TDZ showed necrosis resulting in loss of all explants. A strong genotypic variation was observed among three clones of Sequoia sempervirens for axillary bud break and subsequent shoot elongation over all cytokinins. In another experiment, the optimum concentration of zeatin was determined for the best axillary shoot proliferation system for all three clones. The interaction of zeatin levels and genotypes of Sequoia sempervirens was also investigated. The influence of all these factors, in addition to environmental conditions, on micropropagation of Sequoia sempervirens will be discussed.

P-1101 Clonal Propagation of *Phoenix dactylifera* cv. Barhee via somatic embryogenesis. S. Bhaskaran and R.H. SMITH. Dept. of Soil & Crop Sciences, Texas A&M Univ., College, TX 77843.

Phoenix dactylifera cv. Barhee is an elite variety of date palm, rapid propagation of which is highly desired in the date palm growing regions of the world. Clonal propagation of this cultivar via somatic embryogenesis was achieved from shoot meristem and immature inflorescences of the maternal plant. A high level of 2,4-D is required for callus initiation. Culture of callus on a hormone-free medium results in a friable embryogenic callus, which on transfer to a suspension culture develops into numerous individual embryos in a fairly synchronous manner. This procedure has potential for large scale propagation as well as for encapsulation of embryos for storage.

P-1102 RAPID IN VITRO PROPAGATION OF Kielmeyera coreacea Martius. J.E. PINTO and E.F. Arello. Laboratory of Tissue Culture, ESAL, Lavras, MG, 37200, Brazil.

The Kielmeyera coriacea Martius(Guttiferae) is very commum to the brazilian "cerrado" soils and has been very much explored for its thicker suberized bark, from which, chemicals complex with pharmacological properties such as, OSAJAXANTONA, that has showed eficiency in the schistossomose control.This present work has been trying to study in vitro the behavior that specie, as well as,the viability of its micropropagation. Axillary bud development and adventitious bud forma - tion was obtained with decapitated shoot explants of K. coriacea. The basic culture medium used was Murashige & Skoog (1962) sup - plemented with BAP and NAA and the avaliations were made at 45 days of cultive in vitro. K. coriacea showed higher adventitious bud formation in 5.0 mg BAP/l + 0.1 mgNAA/l. Bud development was obtained in medium with lower BAP concentrations.The optimal tempera tures for shoot growth and development were 27 and 30°C. Maximal rooting of shoots was obtained using shoots with 4.0 cm height and with the culture medium supplied with 4.0 mg IBA/l. In general, the pHs of 5.4 and 5.7 were good for the root system in vitro of K. coriacea.

P-1103 Factors Affecting In Vitro Rooting of *Quercus suber* L. A. ROMANO, C. Noronha and M.A. Martins-Loução. Departamento de Biologia Vegetal. Faculdade de Ciências de Lisboa. Campo Grande. Bloco C2. Piso 4. 1700 Lisboa. Portugal.

Quercus suber L., the primary source of industrial cork, occurs naturally and is cultivated in western Mediterranean region. The mass clonal propagation of selected genotypes is a potentially valuable method for accelerating the improvement of this important species, since conventional asexual methods have been unsuccessful. In order to obtain optimum conditions for in vitro propagation of *Q. suber*, several rooting experiments were performed. We compared the effects of growth regulators (IAA- indol-3-acetic acid, NAA-α-naphthaleneacetic acid and IBA-indole-3-butyric acid) carbohydrate source (sucrose, glucose), light intensity, temperature, addition of activated charcoal and other, on rooting induction. Excised shoots were either cultured on GD medium (Gresshoff and Doy, 1972) supplemented with auxins or dipped in concentrated solutions and subsequently shoots were transferred to GD auxin-free medium. IBA was found to be more suitable for rooting induction. The percentage of rooting increased with increasing concentrations of sugar, being glucose more effective than sucrose. Semi-solid medium is better than liquid medium. Sterilized perlite was tried as an in vitro rooting substrate with promising results. Activated charcoal promotes rooting for high auxin concentration and does not prevent shoot necrosis. 25°C was considered the most suitable temperature for rooting induction. Number of rooted shoots and quality of roots was specially influenced by darkness during the first 7 days of rooting.

P-1104 Shoot Multiplication and Plant Regeneration from Shoot-tip of Gmelina arborea by In Vitro Culture. S.K. ROY, J. Sen and M.S. Islam. Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh.

Apical and axillary shoot bud multiplication and plant regeneration has been acheived in Gmelina arborea using explants from shoot tip of 12-year-old trees, growing in the field. The explants were cultured aseptically on Murashige and Skoog (MS) based regeneration media, with different concentrations & combinations of BAP and NAA. Results showed that addition of BAP at 1.0 mgl^{-1} and NAA at 0.1 mgl^{-1} to MS medium induced maximum number of shoot buds. For inducing axial growth in regenerated bud primordia, the hormone concentration of the medium was lowered and casein hydrolysate at 100 mgl^{-1} and coconut milk at 10% (v/v) were added. For rooting the well developed shoots were excised and implanted onto the rooting medium. Maximum rooting occurred, when shoots were placed on half-strength MS medium containing 1.0 mgl^{-1} each of IBA and IAA, irrespective of dark or light incubation. The percentage of both rooting of shoots and survival of the rooted shoots was 70-80%. Long term continuous trials using explants from the elite trees throughout the year showed that the period between April-June was the best season for collecting the explant source for rapid and increased multiplication of axillary buds.

P-1105 The Acclimatization of In Vitro Cultured Plantlets in the Breeding of Seedless Table Grapes (Vitis vinifera) in South Africa. I.A. TRAUTMANN and P. Burger. Viticultural and Oenological Research Institute, Private Bag X5026, Stellenbosch, 7600, South Africa.

An increasing demand for seedless table grape varieties has focussed the attention on the breeding of new and improved cultivars. In seedless cultivars the embryo aborts several weeks after flowering. Conventional breeding between seedless (male) and seeded (female) parents resulted in only 10 - 15 % seedlessness. The embryo rescue technique, however, enables the breeder to cross seedless parents resulting in a higher percentage seedlessness than the conventional method. Ovules were removed from the developing berries 4 weeks after flowering and cultivated on Nitsch and Nitsch medium. After 12 weeks the embryos were removed from the ovules and allowed to germinate. The acclimatization of these in vitro cultured plantlets, however, posed a problem as high mortality rates were observed. In order to establish a standardized method of acclimatization, a model system was developed investigating a) the conventional method of acclimatization, b) the adapted acclimatization method of Goussard and Wiid (1989), c) the effect of different soil mixtures, d) the effect of different plastic enclosure periods and e) the effect of Vapor Gard (a film forming polymer of x-pinene acting as an anti-transpirant). The conventional method was preferred to the adapted Goussard/Wiid method as the latter proved to be time consuming and labour intensive. Potting mixtures of speedling mix and vermiculite or perlite resulted in 90 - 100 % survival of plantlets. A plastic enclosure period of 9 - 12 days proved to be optimum. The use of Vapor Gard, however, resulted in high survival rates and the plantlets were healthy and normal. The data indicate that Vapor Gard can be used succesfully to ensure a high survival rate of plantlets and could eliminate time consuming and labour intensive acclimatization procedures.

P-1106 In Vitro Response of Excised Kentucky Bluegrass Seed. W. MSIKITA and H.T. Wilkinson. University of Illinois, Department of Plant Pathology, Urbana, IL. 61801.

In an effort to produce somaclonal variants for possible improvement of kentucky bluegrass (Poa pratensis L.), seeds for 11 genotypes were disinfested and separated into embryonic axis and endosperm tissue. The seed pieces were cultured on modified Murashige and Skoog medium supplemented with 6 combinations (0.0/0.0, 0.1/0.0, 0.2/0.1, 0.3/0.2, 0.4/0.3, 0.5/0.4 mg/l) of 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BAP). Embryonic axes developed callus, and embryo-like structures within 5 weeks, while the endosperm tissue was recalcitrant. Callus development and differentiation was on phylogenic lines and growth regulator combination. The best responding genotypes were cultivar 'Aldephi' and breeding line 'STP+P-4' from which 75% of the cultures formed embryo-like structures on medium supplemented with 0.3 mg/l 2,4-D and 0.2 mg/l BAP.

P-1107 Effect of growth regulators on in vitro embryo culture of Carica papaya. Magdy A.I.Alloufa. Laboratory of Plant Physiology, Biology Department, Centro de Biociências, Universidade Federal do Rio Grande do Norte - Natal-RN - BRAZIL

Carica papaya L. zygotic embryos were cultured on sterile basal medium supplemented with IAA, IBA, NAA, 2,4-D,KIN BAP. Compact highly embryogenic calli were obtained from 60-70% of the cultured embryos after several weeks and were subcultured either on the same medium or on modified medium. Somatic embryogenesis occurred on both media. Efficient precocious germination of somatic embryos was dependent on the presence of Indole-3-butyric acid (IBA) and 2-Isopentenyl adenine 2ip.plants have been successfully established.

Key words: Callus growth, somatic embryos, Carica papaya, growth regulators.

P-1108 The Effect of Growth Retardants on Anthocyanin Production in Carrot Cell Suspension Cultures. A. ILAN and D.K. DOUGALL. Department of Botany, The University of Tennessee, Knoxville, TN 37996-1100.

Different cell lines of carrot cell suspension cultures produce different amounts of anthocyanin. Exogenously supplied gibberellic acid (GA) decreases anthocyanin accumulation in carrot cell suspension cultures. We examined the hypothesis that different endogenous levels of GA are the cause of the differences in anthocyanin accumulation among the cell lines. Paclobutrazol at 1.7×10^{-6}M increased anthocyanin accumulation by 27-42% in several high and low accumulating cell lines. These results demonstrate the existence of an active GA biosynthesis in both high and low accumulating cell lines. However, different endogenous GA levels do not appear to account for the differences in anthocyanin accumulation among the cell lines.

Growth retardants which act at different sites along the GA biosynthesis pathway increased anthocyanin accumulation in carrot cell suspension cultures at concentrations which did not inhibit growth. Both ent-kaurene synthetase inhibitors (CCC, AMO-1618 at concentrations of 10^{-6}M - 10^{-8}M and 10^{-5}M - 10^{-6}M respectively) and monooxygenases inhibitors (ancymidol, paclobutrazol, uniconazole and tetcyclacis at 10^{-6}M - 10^{-7}M) increased anthocyanin accumulation.

The inhibitory effect of exogenously supplied GA on anthocyanin production was reversed in part by paclobutrazol. Paclobutrazol at 1.7×10^{-6}M together with GA_3 increased anthocyanin level from 33% of control in GA_3 treated cell suspension to 76% of control. These results suggests that growth retardants affect anthocyanin accumulation in carrot cell suspension cultures via their effect on GA biosynthesis.

P-1109 Natural Products from Hairy Root Cultures of Astragalus species. I. Ionkova[1], Z. B. Hu[2], and A. W. Alfermann. Institut für Entwicklungs- und Molekularbiologie der Pflanzen, Heinrich-Heine-Universität Düsseldorf, 4000 Düsseldorf, FRG. Permanent address: [1]Medical Academy, Institute of Pharmacology and Pharmacy, 1000 Sofia, Bulgaria; [2]Shanghai College of Traditional Chinese Medicine, Shanghai 200032, PRC

Different Astragalus species (Fabaceae) are used as medicinal plants in Bulgaria and China due to their antiviral and immunostimulant activities. Here we report about the initiation of transformed hairy root cultures of Astragalus boeticus, A. hamosus, A. gummifer, A. membranaceus, and A. mongholicus and about the formation of natural products by these cultured roots. Hairy roots were obtained by infection with Agrobacterium rhizogenes strains LBA 9402, ATCC 15834 and TR 105. Significant differences were observed between the transformation ability of various strains of Agrobacterium. To prove the genetic transformation, opines in the roots were identified by electrophoresis. The hairy roots were subcultivated in hormone free MS-medium containing 20 g/l sucrose and 1 g/l casamino acids. The roots were used for chemical analysis after lyophilisation. Preliminary results of polysaccharide and triterpene saponine contents of the different root lines are presented.

P-1110 Diterpenoids in Transformed Root Cultures of Salvia miltiorrhiza. Z. B. HU[1] and A. W. Alfermann. Institut für Entwicklungs- und Molekularbiologie der Pflanzen, Heinrich-Heine-Universität Düsseldorf, D-4000 Düsseldorf 1, FRG. [1]Permanent address: Shanghai College of Traditional Chinese Medicine Shanghai 200032, PRC.

Salvia miltiorrhiza is a famous traditional Chinese medicinal plant. The root of this plant has been widely used in China for the treatment of cardiovascular diseases. Due to the increased demand for this plant, many Salvia species have been screened as possible sources of tanshinones, the active diterpenoid principles of this drug, and studies on cell cultures of this plant have been carried out as well. However, the production of tanshinones by cell cultures was not stable and decreased gradually in successive subcultures. Since hairy root culture shows rapid growth and stable productivity it seems to be much more interesting. We have established transformed root cultures of Salvia miltiorrhiza by infecting pieces of sterile grown plants with Agrobacterium rhizogenes strains LBA 9402, ATCC 15834, TR 105, R 1601, and A 41027. Different strains show various abilities to induce hairy roots on this plant. Tanshinones, such as tanshinone IIA, tanshinone I, and cryptotanshinone were found in the hairy roots as well as in the liquid medium . We have also investigated the regulation of tanshinone biosynthesis in these root cultures and developed a two-stage culture method for more efficient production of these active compounds.

P-1111 Essential Oils in Different Calli of Garden Lovage. S.Y.Zhang.Laboratory of Cell Biology,Lanzhou University,Lanzhou, China.730000

Garden lovage has been used in chinese medicine.The essential oils are important ingredinets in the herb. We analysed the essential oils in different kind of calli---common calli and embryogenic calli with method of Flash Distillation/ Capillar Gas Chromatography. Several important medical compounds are listed in Table below. In the table, A is control and the results were obtained with roots of Garden lovage, B,C,D are common calli and E,F,G are embryogenic calli which were cultured for 10,20 and 35 days separately .It is shown that, the concentration of the compounds vary very much in calli. Both culture time and kinds of calli affected the productivity of essential oils. Calli, as compared with control, possessed lower concentration of mentioned compounds than that in control. Embryogenic calli, as compared with common calli, higher than that in later. As the culture, cuparene and β-ocimene increased, meanwhile, ligustilide and ocimane increase unstably. Percentage of ocimene and ligustilide in embryogenic calli are near that in control, cuparene and butylphthalide are higher than that in conrtol after culturing for 35 days.

Peak No.	Time	Compounds	Peak area (%) A	B	C	D	E	F	G
1	8.6	β-Ocimene	16.1	2.2	2.4	4.1	3.2	2.3	9.2
2	14.4	Ocimene	3.0	1.5	4.2	0.7	4.8	1.5	2.6
3	30.3	Cuparene	2.9	1.2	1.5	4.8	2.4	4.0	3.5
4	46.8	Butylphthalide	0.5	0.1	0.8	0.1	0.4	1.8	2.0
5	49.2	Ligustilide	9.4	0.2	6.3	6.1	5.4	6.8	7.8

P-1112 A New Method for the Selection of Plant Pigment Producing Cells by Flow Sorting
K. SAKAMOTO, K. Iida and T. Koyano
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The flow cytometric method has been extensively used to select targeting cells or determine intracellular substances of animal, bacterial, yeast and plant cells. However, the instrument can be applied to only those having self-fluorescence in the cells or the stained cells by fluorescent reagents. So, this method has not been utilized for the selection of visual pigment producing cells which do not possess fluorescence.We found a novel method to select plant red pigment producing cells by new means of cell staining. After staining with 0.2ppm fluorescein isothiocyanate (FITC) the protoplasts of *Aralia cordata* cultured cells, which were composed of the anthocyanin pigment highly-producing cells and non-producing cells, the protoplasts were observed under a fluorescence microscope. The green fluorescence of FITC from the non-producing cells was clearly ascertained by the naked eye. On the other hand, the fluorescence of the anthocyanin producing cells was completely missing. This phenomenon resulted from compensation of the green fluorescence (λmax 525nm) of FITC and the green light absorption (λmax 525nm) of anthocyanin. Through investigation of the flow cytometric method developed on the basis of this phenomenon, the histograms of these protoplasts expressed the distinction between the anthocyanin producing cells and the non-producing cells. Then, the targeting cells were sorted using this method and the collected anthocyanin producing protoplasts were confirmed by microscopy.

P-1113 Obtaining of Highly Productive Digitalis purpurea Strains for Cardenolides in a Shoot Culture.

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Cardenolides such as digitoxin are used to treat congestive heart failure. Leaves of D.purpurea plant contain 5mg-digitoxin equivalents(DE)/g-dry wt (determined by EIA). However, as far as authors know, DE content in a shoot-forming culture was only 1/20 of that in the plant. A high productive the strain should be necessary for increasing the productivity by a shoot-forming culture. A wide variety wouldn't be found in DE content of individual shoots derived from axillary buds. However, once undifferentiated cells (calli) induced from the shoots, the calli would aquire heterogeneity and result in the regeneration of shoots which had a wide variety of DE content.

We have attempted to obtain highly productive strains using a following method via callus. Shoot tips derived from seedling of D.purpurea was transferred to Murashige & Skoog agar medium containing IAA and BA in darkness. After a week calli were induced, successively etiolated multiple shoots were induced from the calli. The shoots were divided into 20 to 25 segments. Individual strains were transferred into Nitch & Nitch agar medium containing kinetin, and grown for three weeks in light (a light/dark period of 12h/12h). A large difference was shown in DE content, which was analyzed by EIA, among the strains. After repeating the procedure for four times, DE contents of the strains were found to be ranging from 0.1 to 1.4mg/g-dry wt and morphological changes were observed in a part of the strains. These results suggested that highly productive strains could be obtained from the method.

P-1114

Analysis of soluble proteins during somatic embryogenesis in *Codonopsis lanceolata* L.

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To understand protein pattern, protein analysis was carried out during the somatic embryogenesis according to the development. The embryogenic callus was induced on MS containing 2,4-D 0.1, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l respectively. Somatic embryogenesis was effective on 1 and 2 mg/l 2,4-D. If 2,4-D was not added, nonembryogenic callus was developed and plant regeneration was followed.

The soluble proteins of explant nonembryogenic callus, embryogenic callus, somatic embryo(cotyledonary stage) and plantlet was extracted and analysed by one-dimensional electrophoresis(SDS-PAGE). As the result, the amount of soluble protein was remakably variant according to the developmental stages.

The protein contents in nonembryogenic callus was decreased, while embryogenic callus contain higher amount of soluble protein. And the increased protein synthesis was maintained in somatic embryo. In addition, stage specific soluble proteins were characterized according to the development of somatic embryo.

P-1115

WITHDRAWN

P-1116

Transformation of Tomato (*Lycopersicon esculentum L.*) Medialed by *Agrobacterium tumefaciens*

Hsin-Hsiung CHANG and Kuen-Long CHEN
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Transformation of tomato (*Lycopersicon esculentum L.*) mediated by *Agrobacterium tumefaciens* with plasmids carrying neo (neomycin phospho transferase II) gene was conducted. The objectives of this study were to examine the inoculation reaction between *Agrobacterium* strains and host plants, and to discuss the possible factors affecting the transformation efficiency. In addition, the phenolic compounds eluted from wounded tissues was identified and the correlation between those compounds and transformation efficiency was also elucidated.

When various tomato explant was inoculated by LBA 4404 (pBI121), it was found the inoculation of leaf section had the highest transformation frequency. Such transformation frequency was found to be positively correlated with the contents of phenolic compounds eluted from wounded tissues. Both kinds of phenolic compounds — acetosyringone (AS) and sinapinic acid (SA) were found to play an important role in the transformation system. The exogenous addition of 200μMAS during co-cultured period could improve the transformation efficiency. The chimeric genes were confirmed to be integrated into the genomes of transgenic tissues by selection culture, opine assay, NPT II detection and southern hybridization.

In addition, the cointegrated vector, C58C1(PGV 2260::NG1) was found to have better effect on the transformation than the binary vector, LBA4404(PBI 121).

P-1117 Production of transgenic rice plants using *bar* gene as a selectable marker. Keerti S. Rathore, VIJAY K. CHOWDHURY and Thomas K. Hodges. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, U.S.A.

The phosphinothricin acetyl transferase (PAT) gene *bar*, derived from *Streptomyces hygroscopicus*, has been reported to function as a selectable marker to obtain transgenic plants from maize, tobacco, potato, and *Brassica* species and calli from rice. We are assessing its use in obtaining transgenic rice plants. We have made two constructs: pG35*bar*A and pG35*bar*B, each with a 35S promoter and nos polyA tail. In the *bar* gene used for the construction of pG35*bar*B, the sequence immediately preceding the translation start codon had been modified for optimal translation in eukaryotes (White et al., Nucleic Acids Res.18:1062, 1990). Transformation of suspension culture-derived rice (*Oryza sativa*, cv. Nortai and Radon) protoplasts was carried out using PEG-mediated DNA uptake. Gene expression was monitored by analyzing PAT activity using the thin layer chromatography method. Transient gene expression assay on protoplasts, 48 hours after transformation, showed that PAT activity was much higher when pG35*bar*B was used; henceforth, this construct was used to obtain stable transformation. Protoplasts were plated in Kao protoplast regeneration medium on filters over nurse cells for 3-4 weeks followed by transfer of filters to the N6 selection medium containing phosphinothricin. Resistant colonies were visible after 2-4 weeks on the selection medium. PAT assay on the resistant calli confirmed the expression of *bar* gene. Southern blot analysis of the putatively transformed calli showed that *bar* gene had integrated in the genomes of these resistant lines. Several plants have been regenerated from one of the experiments. Transgenic status of these plants has been confirmed by Southern analysis.

P-1118 Transformation and Analysis of Two South African Tobacco Cultivars. S J Hearn, P A KOCH, J R Webster. Division of Food Science and Technology, CSIR, Box 395, Pretoria, South Africa, 0001

Two commercial tobacco cultivars, TL33 and J6 were transformed with the *sul* gene, encoding resistance to the herbicide asulam, and the GUS gene. Image analysis was used to compare asulam resistance of callus from individual transgenic plants on a range of herbicide concentrations. In situ hybridization of transformed and non transformed plants revealed that GUS and *sul* mRNA was mainly associated with meristematically active tissue and glandular hairs. This technique also revealed transgenic plants that were unable to express the relevant characteristics. Histochemical assays for GUS expression showed that GUS acitivity in the flowers of transgenic plants was associated with the floral vascular system and with pollen grains, but not with the developing seeds. The number of T-DNA inserts was estimated from the segregation ratios of herbicide and antibiotic genes by monitoring germination of self-pollinated F1 seeds on MS medium containing asulam (150 mg/l) or kanamycin (100 mg/l).

P-1119 High Expression of a Foreign Gene in Transformed Bean Callus C.I. FRANKLIN, T.N. Trieu, R.A. Dixon, and R.S. Nelson, Plant Biology Division, The Noble Foundation, P.O. Box 2180, Ardmore, OK 73402.

Kanamycin resistant callus was produced from hypocotyl or leaf explants of green bean (*Phaseolus vulgaris* L.) when cultured on a defined medium containing 50 mg/l kanamycin after 3 day co-cultivation with *Agrobacterium tumefaciens* (EHA101 containing the binary vector pKYLX71-GUS). Southern blot analysis confirmed the insertion of the GUS gene into plant DNA and suggests that each transformed callus line resulted from a unique single copy insertion of the T-DNA. The presence of the NPT-II protein in crude cellular extracts of these cells was confirmed by ELISA. GUS expression in the cells from these callus clumps was confirmed by histochemical assay. Preliminary results based on fluorometric assay indicated a very high level of GUS gene expression in the callus. Further work is being conducted to determine whether the high expression is based on higher levels of GUS protein or lower levels of total protein in the callus compared with that found in transgenic plants.

P-1120 **Transformation of Another Solanaceous Species, Datura innoxia Mill. by Direct Gene Transfer and Regeneration of Transformed Plants from Protoplast Derived Calli.** Thilo Schmidt-Rogge, MARTIN MEIXNER and Otto Schieder. Freie Universität Berlin, Institut für Angewandte Genetik, Albrecht-Thaer-Weg 6, 1000 Berlin 33, Germany

Our aim is to develope a gene tagging system with haploid Datura innoxia, because haploids of this solanaceous species are very stable in the greenhouse as well as in tissue culture.

One of the basic needs for this purpose is to establish a transformation/regeneration system which is fast, easy to handle and of high efficiency.

We generated a transformation protocol for haploid protoplasts of Datura innoxia which is a modification of a $MgCl_2$-PEG-electroporation method (NEGRUTIU et al., 1987). In experiments with different plasmids harbouring Hygromycin as well as Kanamycin resistance genes under the control of various plant promoters we obtained relative transformation rates higher than one percent. The integration and expression of the heterologous marker genes was demonstrated by growing on selective media, biochemical detection of NPT-activity as well as Southern blot analysis.

Negrutiu, I., Shillito, R., Potrykus, I., Biasini, G. and F. Sala. Hybrid genes in the analysis of transformation conditions. Plant Molecular Biology : 363-373 (1987)

P-1121 Development of Plant Regeneration and Transformation Systems in Sweet Potato (*Ipomoea batatas*).
J.M.LOWE, C.A.Newell, F.Buitron[1], F.Medina[1], and J.Dodds[1]. Applied Plant Technology Laboratory, Agricultural Genetics Company Limited, Babraham, Cambridge, CB2 4AZ, England. [1]International Potato Centre, La Molina, Apartado 5969, Lima, Peru.

Sweet potato is an important food crop for large regions of the developing world, particularly South East Asia and South America. Problems with reduced yields due to insect pests have resulted in the need for some form of control. As part of an integrated pest management approach, attempts are being made to introduce a trypsin inhibitor gene, isolated from cowpea (CpTI), into in vitro sweet potato explants. Preliminary work has shown that direct plant regeneration is possible from explants of the cultivar Jewel using media containing Murashige and Skoog salts, sucrose, Gelrite, supplemented with zeatin for root pieces, IAA and kinetin for petioles and internode pieces. As the level of regeneration is relatively low (typically 2-20%), research is currently directed towards improving this frequency. Furthermore, the possibility of indirect regeneration (i.e. via a callus and/or root phase) is being investigated. Results from initial transformation experiments using a construct containing CpTI, along with *gus* and *npt*II, in *Agrobacterium tumefaciens* strain LBA4404, indicate that up to 100% of explants can produce transformed callus. Work is presently focussed on the feasibility of obtaining transformed roots and regenerating transformed plants from such roots.

P-1122 Expression of an Abscisic Acid Responsive Promoter in *Picea abies* (L.) Karst. Following Bombardment from an Electric Discharge Particle Accelerator.
R.J. NEWTON[1], H.S. Yibrah[2], Niu Dong[1], D.H. Clapham[2], and S. von Arnold[2]. [1]Department of Forest Science, Texas Agricultural Experiment Station, Texas A&M University System, College Station, TX, 77843-2135. [2]Department of Forest Genetics, Swedish University of Agricultural Sciences, Box 7027, S-750 07 Uppsala, Sweden.

The 1.5 kilobase promoter sequence upstream of Dc8, a late embryo abundant gene of *Daucus*, fused to the reporter β-glucuronidase gene was introduced into several tissues of *Picea abies* via a custom-made electric-discharge particle accelerator. Transient GUS expression was measured histochemically as spot number 2 d after bombardment. Embryogenic suspensions gave higher levels of expression depending upon cell line than embryogenic callus or zygotic embryos. Expression was enhanced when cultures were treated with abscisic acid for 3 d before bombardment. A mean and maximum of 17.0 and 34 spots/disk, respectively, were observed with the best cell line, which was comparable with the level of expression driven by an enhanced 35S promoter.

P-1123 Genetic Transformation of Peanut Callus via *Agrobacterium* Mediated DNA Transfer.
C.I. Franklin, K.M. SHORROSH, B.G. Cassidy and R.S. Nelson, Plant Biology Division, The Noble Foundation, P.O. Box 2180, Ardmore, OK 73402.

Transformed peanut (*Arachis hypogea* c.v. Okrun) callus was produced from hypocotyl explants after 4 day co-cultivation with *Agrobacterium tumefaciens* on a defined medium followed by selection with 200 mg/l kanamycin. Three different *Agrobacterium* strains (EHA101, LBA4404 and ASE1) carrying the binary vector pKYLX71-GUS were tested for their ability to transform peanut hypocotyl tissue. Transformed callus was produced by all three strains tested. Expression of glucuronidase from the chimeric 35S promoter-GUS-Rubisco 3' end construct in the transformed callus was confirmed by histochemical assay for the enzyme. In this report, a protocol for obtaining transformed peanut callus is described and the experimental data to confirm the integration and expression of the foreign gene construct are presented. Also, the use of this callus transformation method to study coat protein-mediated protection against peanut stripe virus is discussed.

P-1124 Direct gene transfer procedures for Eucalyptus genetic transformation.
C. TEULIERES, N. Leborgne and A.M. Boudet. Centre de Biologie et Physiologie Végétales, Université Paul Sabatier, 118 route de Narbonne 31062 Toulouse Cedex, France.

Eucalyptus are fast growing trees of particular interest for pulp industry and their improvement through biotechnology is being actively investigated.

Polyethylene glycol or electrical treatment of *Eucalyptus gunnii* protoplasts derived from callus or cell suspension culture resulted in a transient expression of reporter gene (B glucuronidase). Cell division of these protoplasts can be observed and microcalli can be obtained.

Electrical treatments also allowed a transient expression of GUS gene in intact cells. That is quite unusual and very interesting because in our hands the cell suspension cultures are able to regenerate calli with roots and buds.

Microprojectile bombardment experiments on primary calli using a particle gun are in progress. One potential interest of such a procedure is that calli can regenerate whole plants through organogenesis.

P-1125 Manipulation of Proline in Transgenic Cotton.
N.L. TROLINDER and D.W. Ow. USDA-ARS, Route 3, Box 215, Lubbock, TX 79401 and USDA-ARS, Plant Gene Expression Center, 800 Buchanan, Albany, CA 94710.

Cotton (Gossypium hirsutum L.) was transformed via Agrobacterium to insert a chimeric gene known to regulate the oversynthesis of proline in bacteria. Increased proline levels have long been associated with water and thermal stress in many plants and is believed to have a protective function. Cell cultures derived from tissues of primary transformants have increased resistance to environmental stress. Progeny were tested for the effect of temperature extremes, water, and salinity stress.

P-1126
The Use of Microprojectile Bombardment in the Transformation of Peanut.
J.A. SCHNALL and A.K. Weissinger. Department of Crop Science, North Carolina State University, Raleigh, NC 27695-7620.

In order to design an efficient, reliable transformation protocol, it is essential to maximize the targeting of tissue that will produce fertile plants. Two approaches are currently being undertaken to increase the likelihood of success. First, explants are bombarded and regenerated under selection. Leaflet tissue can easily be induces to form fertile plants in vitro, and can be placed under kanamycin selection. At present, we can routinely generate both non-transformed plants and stably transformed callus tissue, but the production of stably transformed plants has yet to be achieved. Alternatively, embryogenic callus provides a more prolific source for the regeneration of fertile plants. The embryogenic tissue transiently expresses GUS at a very high rate, and can be subjected to selection by hygromycin. The second approach involves targeting germ line tissue in order to produce chimeric plants yielding stably transformed offspring. Whole embryos are rapidly excised from dry seeds, and the apical domes housing germ line cells are easily uncovered for bombardment. The goal of this procedure is to produce chimeric plants, necessitating the use of GUS staining, rather than selective agents, in the recovery of stably transformed progeny.

P-1127 Development of a Haploid Sporophyte Agrobacterium-mediated Transformation System in Brassica napus.
M. ARNOLDO[1,2], N. MacLean[1], B. Huang[1], R. Kemble[1,2]. [1]Allelix Crop Technologies, a Division of Pioneer Hi-bred Production Ltd. 6850 Goreway Drive, Mississauga, Ontario L4V 1P1. [2] University of Toronto, Dept.of Botany, 25 Wilcocks St., Toronto, Ontario. M5S 3B2

With the use of haploid explants as targets for transformation, the introduced gene is fixed in the homozygous condition in the primary transgenic. In contrast, the use of standard diploid tissue requires an additional generation to identify homozygous seed. Thus the use of haploid tissue results in a time savings of 6 and 9 months, for spring and winter type B. napus respectively. We have developed a haploid stem segment transformation system that succeeds in producing haploid transformed shoots with the same efficiency as the counterpart diploid system. Upon chromosome doubling by colchicine treatment, seed was set and collected. No segregation was evident when challenged on kanamycin, thereby confirming homozygosity. In addition, we have developed regeneration protocols for haploid leaves and haploid hypocotyls to be utilized as future targets for transformation.

P-1128
Micro-electroporation: A method of introducing macromolecules into single cells of higher plant tissue
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Micro-electroporation has been proven useful for introducing macromolecules (FITC-IgG) into onion (*Allium cepa*) bulb tissue cells. Micro-electroporation first involved injecting macromolecules into cell vacuoles and then permeabilizing the cell membranes using electric pulses given via a control electrode outside the cell and a microelectrode (the microcapillary) in the vacuole. The microcapillary was the same one used in the micro-injection. The injected macromolecules diffused into the cytoplasm from the vacuole through tiny holes in the tonoplast induced by the electric pulses. The fluorescence of FITC was strongly enhanced when the ambient pH increased from pH 6. Therefore, the movement of FITC-IgG from the vacuole (pH 5 to 6) into the cytoplasm (pH higher than 7) could be clearly identified under a fluorescence microscope. The microelectrodes (or microcapillaries) had an electric resistance of 100 to 150 MΩ and a diameter of 5 to 7 μm at the tip. The number of the permeabilized cells increased as the differential voltage for the electric pulses increased higher than 3 volts across the tonoplast. However, the percentage of surviving cells was apparently reduced when the differential voltage was higher than 6 volts. Similarly, the number of permeabilized cells increased sharply when the number of electric pulses was above 3, but there were fewer surviving cells. The desirable number of electric pulses was 5. This method was helpful to avoid the obstacle of cell walls to the movement of macromolecules and made it easier to introduce macromolecules into a specific cell than ordinary micro-injection.

P-1129

Transformation and Analysis of Inducible PAL Genes in Potato. F.-F. LIN, J. Collier, L. Fitzmaurice and E.L. Virts, SIBIA, 505 Coast Boulevard South, La Jolla, CA 92037.

Disease resistance in plants involves inducible defense responses including accumulation of phytoalexins and hydroxyproline-rich glycoproteins, deposition of lignin, and increased activity of certain hydrolytic enzymes. L-phenylalanine ammonia-lyase (PAL) is an enzyme which catalyzes the first step in the biosynthesis of one class of phytoalexins, the phenylpropanoids. Transcription of the PAL genes is activated by a wide range of stimuli including wounding, glycan elicitors, and the reduced form of glutathione. Two inducible PAL promoters isolated from Solanum tuberosum cv. Desirée have been fused to a reporter gene [E. coli betaglucuronidase (GUS)] and introduced into potato (S. tuberosum cv. Desirée) by Agrobacterium-mediated transformation using tuber discs. Approximately, 150 kanamycin-resistant transgenic potato plants were obtained. The assays for betaglucuronidase activity confirmed that the GUS gene is stably integrated into the S. tuberosum genome and that the PAL promoters are functioning in these transgenic plants. The inducibility of the promoters will be tested in the whole plants, and the results of this analysis will be presented.

P-1130 Expression of *gus*A Gene with an Intron in Sweet Potato and Garden Egg Plant. A. POROBO-DESSAI, E. T. Blay, C. S. Prakash and K. Nakamura*. Plant Molecular and Cellular Genetics Lab, Tuskegee University, Tuskegee, AL 36088, USA. *Lab of Biochemistry, School of Agriculture, Nagoya University, Chikusa-ku, Nagoya, 464-01, Japan.

The reporter *gus*A gene with an intron in its coding sequence was efficiently expressed in sweet potato (*Ipomoea batatas*) and garden egg plant (*Solanum integrifolium*), a popular vegetable in West Africa. The disarmed *Agrobacterium tumefaciens* EHA 101 with a binary vector pIG 121-Hm containing a modified intron from the castor bean catalase gene was employed. The intron sequence was placed within the N-terminal part of the ß-glucuronidase (GUS) coding region fused to a CaMV 35S promoter. When cocultivated with a variety of explants, a very high expression of the *gus*A gene was detected histochemically. This indicates that the intron from the castor bean catalase gene was spliced efficiently in the cells of sweet potato and garden egg plant. The frequency and extent of transformation varied with the type of explant. There was no detectable expression of the *gus*A gene in the bacteria. Thus, the use of this reporter gene with the intron eliminates the problem of false positives and aids early detection of transformed tissues. The pIG 121-Hm also contains two antibiotic resistance genes, *npt II* and *hpt*. The selective proliferation of transformed tissues was achieved by culture of explants on MS medium supplemented with kanamycin and hygromycin.

P-1131

The Effects of Ultrasound on Uptake of Calcein and DNA by Protoplasts and Cells of Kentucky Bluegrass. H. TANG, G. Jelenkovic and C. Chin. Dept. of Horticulture, Rutgers University, New Brunswick, NJ 08903.

The effects of ultrasound on uptake of foreign substances by protoplasts and suspension culture cells of bluegrass were studied using calcein (3,6-dihydroxy-2,4-bis-[N,N'-di-(carboxymethyl)-aminoethyl]fluoran). Uptake of calcein was monitored with a fluorescence microscope. Neither the protoplasts nor cells took up calcein but treatment with ultrasound facilitated the uptake. Pronounced stimulation of uptake was observed with protoplasts but only moderate stimulation was observed with cells. The ultrasound facilitated uptake was dependent on sound frequency, power output and duration of treatment. Uptake stimulation increased with treatment time but prolonged treatment caused the rupture of protoplasts. Other factors including temperature, osmoticum in the medium and dimethyl-sulfoxide also affected ultrasound stimulated uptake.

Ultrasound also facilitated uptake of plasmid DNA by protoplasts and cells of bluegrass. In this study a plasmid pBI 121.2 carrying a reporter gene - glucuronidase (GUS) was used. Transient expression of this gene was detected.

Protoplasts and cells subjected to ultrasound treatment divided and formed microcalli in agarose medium.

P-1132 Maize Haploid Protoplast Transformation and Regeneration of Transgenic Plants. K. SUKHAPINDA[1], ME. Kozuch[1], B. Rubin-Wilson[1], KA. Smith[1], KP. Crawford[1], WM. Ainley[1], JC. Mitchell[2], DJ. Merlo[1]. [1]DowElanco Biotechnology Laboratory, Midland, MI 48674, [2]DowElanco Champaign Laboratory, Champaign, IL 61820

Protoplasts were isolated from maize haploid cell lines derived from microspore cultures. DNA of a plasmid containing genes encoding ß-glucuronidase (GUS) and neomycin phosphotransferase II (NPT II) was introduced into the protoplasts by electroporation. Another gene of interest located on an independent plasmid was coelectroporated. Several stably transformed callus isolates were recovered on selection media. Enzyme assays and DNA analyses using Polymerase Chain Reaction or Southern blot techniques confirmed the presence of the gene or genes in the transformed isolates. Approximately 80% of the callus isolates analyzed contained the selectable marker gene (NPT II), 50% of these contained the linked gene (GUS), and 10% contained the unlinked gene of interest. Plants regenerated from some of the transformed callus tissue showed the marker enzyme activities as well as the presence of the marker gene. Chromosome examinations performed on representative plants showed that they were haploid. As expected, these plants were infertile. Chromosome doubling techniques are being employed to develop fertile diploid transgenic plants.

P-1133 Transformation of *Lycopersicon esculentum* with an ACC Deaminase Gene to Inhibit Ethylene Production. S. Vanderpan, V. Ursin, R. Sheehy, W. Hiatt. Calgene, Inc. 1920 Fifth Street, Davis, CA 95616 USA.

The plant hormone ethylene is produced by tomato fruit and promotes ripening. Through *Agrobacterium* mediated transformation we have introduced a *Pseudomonas* gene encoding 1-aminocyclopropane-1-carboxylate (ACC) deaminase into tomato, creating a new branch point in the ethylene biosynthetic pathway. ACC deaminase metabolizes ACC, the immediate precursor to ethylene, to ammonia and α-ketobutyrate. The analysis of tomato plants expressing ACC deaminase and the effect of expression on ethylene production will be discussed.

P-1134 Transformation of an Elite Maize Inbred Through Microprojectile Bombardment of Regenerable, Embryogenic Callus. K. AVES[1], D. Genovesi[1], N. Willetts[2], S. Zachwieja[2], M. Mann[2], T. Spencer[2], C. Flick[2] and W. Gordon-Kamm[2], DEKALB Plant Genetics; [1]3100 Sycamore Rd., DeKalb, IL. 60115; [2]Eastern Point Rd., Groton, Ct. 06340

An early embryogenic Type II callus culture was established from an elite maize inbred (designated AT824). This callus has a characteristic bright yellow color and a friable, granular consistency. The callus was readily dispersed onto filters for microprojectile bombardment with DNA-precipitated gold particles. Using a combination of maize genes that regulate anthocyanin synthesis (*C1* and *B*) or the *E. coli* gene encoding the GUS enzyme, a range of 300 to 3000 transiently expressing foci were observed per bombarded filter, over numerous experiments. Subsequently, bialaphos selection was used to recover *bar*-expressing colonies. Phosphinothricin acetyltransferase activity (which confers bialaphos resistance) was confirmed in these embryogenic transformants, as was the presence of the *bar* gene through PCR and Southern blot analysis. Regeneration is in progress and numerous plantlets have been recovered.

P-1135 Isolation of Immature Inflorescence and Ear Protoplasts and Their Culture in Cereals. X.Y. CHENG. Institute of Nuclear Agricultural Sciences, Zhejiang Agric. University, Hangzhou 310029, CHINA

A general procedure has been developed to isolate enzymatically protoplasts from immature inflorescences of *Triticum* and *Oryza sativa* and immature ears of *Zea mays* as well as inflorescences of other cereals. The yields of the protoplasts isolated from one gram of the tissues were over $5X10^6$ in corn and over $1X10^7$ in the other cereals, thus ensuring easy recovery of meristematic cells for both physiological and genetic studies in cereals. The isolated protoplasts were cultured in a number of media for induction of further development. Cell wall generation, cell division and colony formation have been observed in most of the species tested with varied frequencies. Colonies consisting of up to 20 cells in several Triticum species, up to 7 cells in rices and more than 100 cells in corns have been observed in chemically defined media without nursing cultures. Further approaches to improve the culture response of the protoplasts as alternative sources for in vitro cloning of plants are discussed.

P-1136 Development of Glyphosate as a Selectable Marker for the Production of Fertile Transgenic Corn Plants. A. HOWE, F. Tamayo, S. Brown, C. Armstrong, M. Fromm, J. Hart, S. Padgette, G. Parker, R. Horsch. Monsanto, 700 Chesterfield Parkway North, Chesterfield, MO 63198

Development of an efficient and reproducible selectable marker for the production of fertile transgenic plants is an essential step in developing a commercially viable transformation system. Published reports have documented the use of the ALS or BAR genes for selection and recovery of transgenic corn plants. This poster describes the development of glyphosate as a selectable marker for the recovery of transgenic embryogenic corn callus and the production of plants tolerant to Roundup herbicide. We have developed a system in which transgenic callus is reproducibly recovered following glyphosate selection. Plants have been regenerated both on and off glyphosate selection media, and are tolerant to normally lethal levels of the herbicide. Both sprayed and unsprayed plants are male and female fertile. Seed set from both self and outcross pollinations from regenerated plants has been excellent. R1 segregation tests are in progress.

P-1137 Expression of an ABA Responsive Promoter in *Pinus elliottii* Following Gas-Driven Particle Bombardment. NIU DONG' and R. J. Newton . Department of Forest Science, Texas Agricultural Experiment Station, Texas A&M University System, College Station, TX 77843-2135.

The 1530 bp promoter sequence upstream of Dc8, a late embryo abundant gene of *Daucus*, fused to the reporter GUS gene was introduced into embryogenic cultures of slash pine (*Pinus elliottii*) via tungsten and gold particle bombardment. Transient expression was measured histochemically as blue spot number 2 d after bombardment. Expression was enhanced more than twice when cultures were treated with abscisic acid for 7 d before bombardment. The maximum expression of Dc8 was 1/2 of that observed with the CaMV 35S promoter.

P-1138 Biochemical, Histological, and Molecular Characterization of Haustorial Development in Root Cultures of *Striga asiatica*. S.J. WOLF and M.P. Timko. Department of Biology, University of Virginia, Charlottesville, VA 22901

Striga asiatica, commonly called witchweed, is a root parasite of important agronomic crops such as corn and sorghum. *Striga* attaches to its host root by means of a specialized organ called a haustorium. Past studies of haustorial development have used intact radicles of *Striga* seedlings. We have recently reported the development of a novel system for the study of haustorial formation in *Striga asiatica* which used in vitro root culture (Plant Science 73:233-242). Cultured roots form haustoria following exposure to 2,6 dimethoxy-*p*-benzoquinone (2,6-DMBQ), a haustorial induction factor isolated from host root extracts. Haustoria formed on cultured roots appeared morphologically similar to those formed by *Striga* radicles. Major potential advantages of our root culture system over the use of radicles are that root culture haustoria are larger and free of contaminating organisms. In this study we provide additional evidence that haustoria formed on in vitro root cultures are comparible to those formed on intact radicles. Haustorial development from the radicle meristem in seedlings is a rapid process. Upon induction, root elongation stops abruptly, rapid cellular differentiation is initiated, a localized expansion of cortical cells occurs, and surface haustorial hairs develop. Two-dimensional in vivo protein profiles of root culture haustoria were compared to protein profiles of radicle haustoria. These studies showed that the same 2,6-DMBQ-induced proteins existed in root culture and radicle haustoria. A time course study of root culture haustoria demonstrated that at least four major protein changes occured following exposure to 2,6-DMBQ. Three of those proteins increased in abundance as early as two hours after exposure to 2,6-DMBQ. Histological studies of haustorial development in root cultures showed similar cellular changes as those reported for radicles such as the expansion of cortical cells, the emergence of haustorial hairs, and the development of densely stained cells at the haustorial apex. As a step toward studying changes in gene-expression during haustorial formation we have used the DNA polymerase chain reaction to isolate partial cDNA and genomic clones encoding several different cytoskeletal actin genes. Actins are encoded by multigene families and tissue-specific function and regulation of the different plant actin genes have been proposed. Southern blot analysis has suggested that at least five actin genes exist within the *Striga* genome. Expression of *Striga* actin genes during haustorial development was studied using Northern blot analysis.

P-1139 Analysis of DNA Polymorphism by Random Amplified Polymorphic DNA Markers in Anther Culture Derived Broccoli Plants. F.-S. Wu and C.-L. LAN*. Department of Biology, Virginia Commonwealth University, Richmond, VA 23284. *Department of Biology, Fu-Jen Catholic University, Taipei, Taiwan, R.O.C.

Anthers from field-grown Brassica oleracea L. ssp. Italica were harvested and pretreated at 35°C for 1-2 days before they were cultured at 25°C in several different culture media for plant regeneration. Embryos formed in the culture were removed and transferred to new culture media and were subsequently regenerated into plants. Regenerated plants were propagated and maintained in vitro by subculturing bimonthly. DNAs were isolated from regenerated plants and were served as the templates in polymerase chain reactions (PCR) using a variety of oligodeoxynucleotide primers as DNA markers. DNA fragments obtained after PCR amplification were analyzed by agarose gel electrophoresis to reveal the DNA polymorphism among the anther culture derived plants and green house grown plants. The differences among the random oligonucleotide primers were compared.

P-1140 Transient Gene Expression in Electroporated Medicago Protoplasts G.P. KOSTURKOVA, M.R. Davey*
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Univ of Nottingham, Nottingham NG72RD,UK*

A non commercial DC square wave generator was used to induce direct uptake of plasmid (pDW2) DNA by protplasts isolated from leaf mesophyll (p-derived clone G-156 from M.varia cv Rambler) and from cotyledons (M.sativa cv Rangelander).

Single or 2-4 consecutive pulses of 0.05-3 ms were applied to 0.4 ml aliquots of protoplast - DNA suspension at field strength of 500, 750 and 1000 V/cm. Electroporated protoplasts were washed and cultivated in KP8 medium for 2 days.

CAT assey was performed to determine the levels of mono- and di-acetylated forms of chloramphenicol for each set of electroporation parameters. Generally mesophyll protoplasts had higher viability and in most of the cases higher CAT activity. Single or multipple pulses shorter than 0.25ms were not effective. Electroporation with single or multipple pulses of 0.75 - 1.5 ms at 750 V/cm or with tripples of 1.0 - 3.0 ms at 500 V/cm brought to highest enzyme activity per a single protoplast what in some of the cases did not correlate positively with high viability. Best compromise of high gene expression (80-99% of total acetylation) and high viability (50%-70%) was achieved following electropulsing at 500 V/cm 3 x 1.0ms or 4 x 0.25ms and at 750 V/cm 1 x 0.75 ms or 1 x 1.5 ms. These parameters used in the experiments for stable transformation gave good results.

P-1141 Expression of a Cyclophilin:GUS Chimeric Gene in Transgenic Rice. WALLACE G. BUCHHOLZ, Jonathan W. Seay, Richard T. DeRose and Timothy C. Hall. Dept. of Biology, Texas A&M University, College Station, Texas 77843-3258.

Cyclophilin, an abundantly expressed protein found in all eucaryotes investigated, has peptidyl-prolyl *cis-trans* isomerase and protein folding activity in vitro and is thought to be involved in protein folding in vivo. As an initial step toward investigating the regulation and function of cyclophilin in plants, genomic and cDNA clones were isolated from rice (*Oriza sativa.*). Comparison of the encoded amino acid sequence with those of maize and human T-cell cyclophilins yielded over 70% identity. Whereas a single cyclophilin mRNA transcript is expressed in other organisms investigated, northern blot analysis of rice yielded two highly expressed transcripts of approximately 800 and 900 nt. The 800 nt band was seen only in root mRNA whereas the 900 nt band was present in both root and leaf mRNA. Southern blot analysis suggests the presence of a small (6 to 8 member) divergent cyclophilin gene family in rice. A chimeric gene was constructed by fusing a 2.5 kb genomic DNA fragment from upstream of the cyclophilin coding sequence to a *ß-glucuronidase* reporter gene. Transgenic rice plants containing the chimeric gene were produced. Histochemical staining of transgenic tissues indicated detectable levels of GUS were expressed only in meristematic and early differentiating cells.

P-1142 Isolation of Triosephosphate Isomerase Gene from Rice. Y. XU and T. C. Hall. Department of Biology, Texas A&M University, College Station, TX 77843-3258

Triosephosphate isomerase (TPI) catalyzes the inter-conversion of dihydroxy-acetone phosphate and D-glyceraldehyde-3-phosphate, an essential isomerization reaction in the glycolytic pathway. In addition, the enzyme plays important roles in other triosephosphate-involving metabolic pathways in plants such as gluconeogenesis, fatty acid biosynthesis, pentosephosphate pathway, and photosynthetic carbon dioxide fixation. As an ancient, ubiquitous, highly conserved protein, TPIs from bacterial, fungal and animal systems have been studied extensively. However relatively little information is available on higher plant TPI. The only gene sequence available for higher plants being from maize and, as yet, no report on plant TPI gene regulation has appeared. Towards the goals of using TPI gene both as a marker for gene evolution and for comparison of the house-keeping gene regulation with that of facultative genes in a homologous system, we have isolated and characterized a cDNA clone and the corresponding genomic clone encoding TPI from rice. The cDNA clone (pRTPI-6) contained an open reading frame of 759 bp, encoding a polypeptide chain of 253 amino acid residues (*Mr* 27,060) that showed 85% nucleotide sequence and 89% amino acid sequence identical match with the maize TPI mRNA sequence. Genomic DNA blot analysis using the cDNA as a probe showed that a single copy TPI gene was present in the rice genome. TPI gene expression in rice was found to be almost ubiquitous. A single mRNA species of 1100 nt was detected by RNA gel blot analysis of RNA isolated from roots, culms, green and etiolated leafs of rice seedlings, but no TPI message was found in the seeds. The genomic clone for the rice TPI has also been isolated.

P-1143 Stable Transformation of Sweet Potato by Electroporation. M. NISHIGUCHI, Y. Uehara and K. Komaki. Lab. of Plant Biotechnology, Dept. of Crop Breeding, Kyushu National Agricultural Experiment Station, 2421 Suya, Nishigoushi, Kikuchi-gun, Kumamoto 861-11, Japan

Electoroporation (EP) has been used to transfer DNA /RNA into plant protoplasts. This method is very efficient and reproducible. We used EP to introduce hygromycin phosphotransferase gene (hph) gene into protoplasts of sweet potato and obtained transformed calli. Protoplasts were isolated from suspension cultured cells. The protoplasts pellet (4 x 10^5/ml) was resuspended in an EP buffer (0.8 ml) containing two types of plasmids (20 µg/ml each) which confer hph or GUS gene. The mixture of protoplasts and the plasmid DNAs was subjected to a 10 msec exponentially decaying pulse delivered by a capacitor (100 µFD) at the field strength of 0.75 kV/cm. After washing the mixture, the EP buffer was replaced by by a MS based culture medium. The protoplasts were cultured for about 3 months while the fresh medium was added monthly. Developed clonies were further cultured in an agarose medium in the presence of hygromycin (20/40 µg/ml). The hygromycin resistant calli were obtained. The DNA was isolated from the calli, digested with restriction enzymes and subjected to gel electrophoresis. Southern hybridization was performed using the probe of the hph gene. It was confirmed that the DNAs from these calli hybridized with the probe. Under the conditions used, the frequency of transformation was estimated to be approximately 1% when the number of colonies was compared in the presence or absence of hygromycin.

P-1144 Regeneration of Kenaf, Hibiscus cannabinus L. plants from explants and tissue cultures. H. SAMARTZIDOU, S. Venketeswaran, N. Kletzly and H. Nguyen. Department of Biology, University of Houston, Houston, TX 77204.

Tissue cultures were established on three Kenaf varieties, *K Gautemala 45, K Gautemala 4 & Salvarodean* from 2-6 mm. segments of hypocotyl, stems, leaf discs of young plants and embryos from aseptic germinated seedlings. A modified Murashige & Skoog medium (MS) supplemented with different auxins and cytokinins alone or in various combinations and concentrations was used. Callus initiation and growth occurred with 2 mg/l of 2,4-dichlorophenoxy- acetic acid (2,4-D) alone or incombination with 0.1 mg/l of α-naphthalene acetic acid (NAA) and/or different concentrations of Benzylamino purine (BAP). Root and shoot initiation occurred with various other combinations. Young plantlets regenerated from callus and other explants. They were successfully transferred eventually to pots and transferred to the greenhouse. The two *Gautemalan* varieties showed best response whereas the *Salvadorean var.* grew slow. A routine method of regeneration of plants is a pre-requisite for other biotechnological applications. Efforts to obtain regeneration in other varieities are pursued. (Supported in part by University of Houston Coastal Center and Komotini Paper Mill S.A., Kavala , Greece).

P-1145

Protein synthesis during carrot embryogenesis and germination: Comparison of zygotic, somatic and DFMA-treated somatic embryos. R.P. FEIRER and P.W. Simon. Biology Department, St. Norbert College, De Pere, WI 54115 (RPF) and USDA-ARS, Department of Horticulture, University of Wisconsin, Madison, WI 53706 (PWS)

Proteins were extracted from zygotic carrot (*Daucus carota* L.) embryos at various stages of development and germination. Significant changes in the proteins were observed as zygotic embryos developed; some proteins were found only in immature embryos, others appeared during germination, and many were found in all stages of development. The protein profiles observed in the developing zygotic tissues were then used as a baseline against which to compare the proteins isolated from tissues undergoing somatic embryogenesis. Embryogenic carrot calli contained several of the transient proteins as well as many of the constitutive proteins found in developing zygotic embryos. Proteins isolated from somatic embryos of different size classes and times of incubation were also examined. While several proteins transiently expressed in zygotic embryos were present, changes in the protein profiles observed in developing somatic embryos were not as substantial as those in developing zygotic embryos. Embryogenic calli and somatic embryos most closely resembled imbibed zygotic embryos, suggesting that carrot somatic embryogenesis might better serve as a model of embryo maturation and germination rather than embryo initiation or early development. The compound difluoromethylarginine (DFMA), known to inhibit somatic embryo development in carrot via inhibition of polyamine biosynthesis, led to changes in the proteins present in the callus tissues. Reduction in the levels of several embryo specific proteins were noted in the DFMA-treated cultures. Both the synthesis of these proteins and embryo development were restored by the presence of putrescine in the culture medium.

P-1146 Somatic Embryogenesis and Plant Regeneration in Zoysiagrasses (*Zoysia* spp.).

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Somatic embryogenesis has been reported in *Zoysia japonica* (L.) Steud, but we found that the developmental stages of explants are critical in forming embrygenic callus. To establish an embryogenic callus culture method, immature inflorescences of various lengths (<0.5, 0.6-10, 11-15, 16-20, >20 mm) or embryos at different maturities (1, 2, 3, 4, 6 weeks after pollination) were cultured on MS or N_6 agar medium supplemented with 2 mg/l of 2,4-D. The most embryogenic calli were produced from immature inflorescences less than 5 mm or from 3-week-old immature embryos with 2 mg/l of 2,4-D regardless of media used. The 2,4-D requirement could be lowered to 1 mg/l by adding 20 g/l of sorbitol to produce and maintain the embryogenic cultures. The culture method was also effective in *Z. sinica* and *Z. macrostachia* X *Z. japonica*. When the callus lines were cultured in the liquid MS medium with agitation, some proliferated rapidly as compact cell clumps of proembryo body. Numerous plantlets regenerated simultaneously upon transfer to auxin-free MS medium. These typical embryogenic cells are cytoplasm-rich and are being used for electroporation-mediated gene transformation experiments.

P-1147

Somatic Embryogenesis of High Latitude-adapted Soybean Cultivars and Germplasms. L. TIAN, D.C.W. Brown, and J. Webb*. Plant Research Centre, Agriculture Canada, Ottawa, Ontario, Canada. K1A 0C6. *Department of Biology, Carleton University, Ottawa, Ontario, Canada. K1S 5B6

High latitude-adapted soybean cultivars and germplasms have been developed relatively recently and are unique for their daylength-insensitivity. We have been characterizing and optimizing their in vitro responses and regeneration capabilities. Twenty soybean lines adapted for a latitude of 45^0 North were investigated for their ability to undergo somatic embryogenesis from immature cotyledon explants. Genotype proved to be a major factor in the ability of cultivars to undergo somatic embryogenesis. Although all the cultivars investigated showed signs of somatic embryogenesis, large variation was observed in their responses. The genetic background of the cultivars which show better in vitro response are being investigated to determine if predictions of regenerative capacity can be made on the basis of pedigree for these germplasm sources. An efficient regeneration system for these germplasm sources is being developed. 2,4-D concentrations and different induction time periods have been evaluated. A high 2,4-D concentration (up to 50 mg/L) with a ten day induction period appears to be optimum for embryogenesis in this germplasm type.

P-1148 Induction of Desiccation Tolerance in Cultured Immature Embryos of Wheat.

D.C.W. BROWN, C.J. Ledderhof and J.A. Simmonds. Agriculture Canada, Plant Research Centre, Ottawa, Ontario, Canada K1A 0C6

Immature embryos (10-21 day post anthesis) of wheat (Triticum aestivum cv. Glenlea) were isolated and cultured on basal Murashige and Skoog medium. Embryos germinated at about 85% frequency in 7 to 14 days, where as the seed stock showed a 90% germination rate. Medium supplied abscisic acid at 10^{-5}-10^{-3}M could inhibit germination by 100% and induced tolerance to desiccation. Slow desiccation over an 8 day period, in plastic Petri dishes enclosed in plastic chambers with controlled relative humidity levels, reduced moisture content to about 7.5% and resulted in the death of embryos not exposed to abscisic acid. Exposure to 10^{-5}M abscisic acid for 3 to 7 days resulted in up to 100% embryo viability after rehydration on basal Murashige and Skoog medium. Induction of desiccation tolerance was found to be dependant on embryo age and abscisic acid concentration and exposure time. Recovered plants were stunted, had reduced internode length and a reduced seed yield, however, selfed progeny of these R_1 plants were similar in appearance to non-tissue culture treated controls. Desiccated embryos could be stored for long periods with no significant loss of viability.

P-1149

Screening of Medicago trautvettezi and Medicago falcata germplasm for somatic embryogenesis. E. SVANBAEV, I. Zadorozhnaya, E. Dzhangalina, D.C.W. Brown*. Institute of Botany, Alma-Ata, Kazakhstan. *Agriculture Canada, PRC, Ottawa, Canada K1A 0C6

The germplasm of Medicago trautvettezi Sumn. originating in different regions of Kazakhstan was screened for its ability to produce somatic embryos on callus tissues derived from cotyledon and hypocotyl explants in four different medium protocols. The highest regeneration frequency was obtained from 5 of 11 accessions using a medium protocol that included cultivation of explants on B_5 medium supplemented with 2,4D (5 mg/l) and kinetin (0.5 mg/l). Embryos development occurred after transferring the callus tissues to MS medium lacking growth regulators. There was no correlation between callus proliferation and embryo production. Regenerable genotypes were identified in 16 of 29 wild accessions of M. falcata. The best response interms of somatic embryo production was obtained from callus tissues derived from cotyledons. The in vitro responses of M. tracetvetteri and M. falcata were comparable with those observed in the closely related species, M. sativa. Experiments are being extended to develop artificial seed technology for propagation and preservation of these wild species. Research was supported in part by an NSERC grant to D.C.W.B.

P-1150 Involvement of glutamate dehydrogenase in induction of alfalfa somatic embryogenesis. K.I. FINSTAD[1,2], D.C.W. Brown[1,2] and K.W. Joy[2]. [1]Plant Research Centre, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6; [2]Dept. of Biol., Carleton University, Ottawa, Ontario, Canada K1S 5B6.

The relationship between ammonium assimilation and plant somatic embryogenesis is indicated by the general requirement of embryogenesis induction for reduced nitrogen, and by reported changes in glutamate dehydrogenase (GDH) activity and isozyme pattern associated with embryogenesis. The nature of this relationship is poorly understood. GDH is particularly interesting because, though ubiquitous throughout the plant kingdom, its function is unclear. Ammonium is thought to be normally assimilated through glutamine synthetase (GS) and not GDH. Extracts from suspension callus cultured with the embryogenesis-inducing auxin 2,4-dichlorophenoxyacetic acid (2,4-D) were compared with those cultured with the non-embryogenic auxin phenylacetic acid (PAA). GDH isozyme profiles were variable but apparently unrelated to embryogenesis. Cultures grown with 2,4-D showed significantly higher GDH activity, lower levels of cellular ammonium, lower accumulation of asparagine and somewhat lower GS activity than cultures grown with PAA. These data suggest that exposure to the embryogenic auxin 2,4-D is associated with a shift from the GS/GOGAT pathway of ammonium assimilation to a pathway involving GDH.

P-1151

Induction of Callusogenesis and Organogenesis in Mature Seeds of Triticum aestivum L. N.A. ZAGORSKA and V. Ilcheva. Institute of Genetics, Sofia 1113, Bulgaria.

Tissue cultures in wheat find an application for genetic investigations and for enriching the genefund of this economically important crop. Successful regeneration of wheat plants is realized when immature embryos or meristematic tissues serve as explants. The work with mature embryos is more difficult but has many advantages - the most important is that they can be used all over the year. The aim of the present investigation was to create a culture medium favouring the callusogenesis and to reveal the morphogenetic potential of the different genotypes. The investigations proved that the concentration of macro salts, saccharose and 2,4-D had an effect on callusogenesis of mature seeds of Tr. aestivum.Most suitable for induction of callusogenesis and enhancement of callus growth rate was the medium of Murashige and Scoog (1962) +5 mg/l 2,4-D. BAP in a concentration of 2 mg/l contributed to the production of highest percentage of calluses with regenerants. The ratio between cytokinins and auxins in the nutrient medium was also of great importance. Highest percentage of regenerating callusses was observed at a BAP:IAA ratio of 10:1 The genotypes tested had considerable differences in their morphogenetic potential. Best regenerating ability was assessed in the cultivars Pobeda and Gladiator. A positive correlation was observed in them between callus growth rate and regenerating ability.

P-1152 **Two Novel Auxin Substitutes for Plant Tissue Culture.** ***J.PONSAMUEL and P.Dayanandan. Lab of Plant Cell and Tissue Culture, Department of Botany, Madras Christian College, Madras-600 059, India.**

***Lehrstuhl Für Pflanzenphysiologie, Postfach 102148, W-4630 Bochum, Federal Republic of Germany.**

Tetraphenylboron (TPB) and Phenylboronic acid (PBOA) are organoborones with auxin-like growth activities. A detailed study was carried out to establish their potential in triggering in-vitro morphogenesis. TPB and PBOA induced callus between 0.1 μm and 100 μm in tuber explants of Daucus carota, cotyledonary explants of Vicia faba, Arachis hypogaea and Anacardium occidentale, leaf explants of Arachis, Coleus parviflours and both leaf and floral axis of Nicotiana plumbaginifolia and on the secondary cortical tissues of Crescentia cujute. Direct rhizogenesis was observed in the cotyledon derived callus of Anacardium at 10 μm. Caulogenesis was observed at 10 μm after 15 d of inoculation when supplied with BAP/2iP at 1 μM. Somatic embryoids were induced in Camellia sinensis on the cotyledonary explants at 10 μM PBOA+1 μM BAP + 10% coconut milk after 40 d in culture. Embryoids were induced on the callus of Coleus by the above treatment after 30 d in culture. TPB/PBOA could be substituted for auxins in the root cultures of Solanum esculentum, Raphanus sativus and Abelmoscus esculentus between 0.01 μM and 10 μM. TPB/PBOA at 10 μM induced cell division leading to the establishment of colonies of mesophyl protoplasts of Arachis. TPB/PBOA also enhanced aminoacid secretion from calcium alginate immobilised cells of Arachis 165% and 138% over control. TPB/PBOA promoted 81% and 163% increase in biomass of Chlorella vulgaris cultured in a bioreactor. The morphogenetic responses brought about by TPB and PBOA are auxin-like. They are more efficient than NAA and 2,4-D. We feel that TPB and PBOA are useful in tissue culture studies where they may not only substitute for auxins but be morphogenetically active in species where other known auxins are inactive.

P-1153 Some Biochemical Changes during Callusing and Differentiation of Tree Species In Vitro.

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Three economically important tree species viz. *Dalbergia lanceolaria, Pterocarpus marsupium* and *Cajanus cajan* were subjected to comparative biochemical analyses during callus development and organogenesis in vitro. The protein content was more in the regenerating calli than in the proliferating calli whilst the total free aminoacids (and proline in particular) followed the reverse trend suggesting an increased protein synthesis during organogenesis. The concentration of total sugar was higher in the developing calli whilst reducing sugar content was more in the regenerating calli-interpretable by an enhanced energy demand associated with the process of regeneration. Perhaps before the onset of organogenesis, the reserve endogenous non-reducing sugars hydrolyse to the reducing sugars, the latter to act as respiratory substrates. The regenerating calli contained relatively more ascorbic acid, the latter being known to provide anti-oxidant protection to an actively growing living system thereby generating a reducing potential for the organisation of life. The aforementioned trend was not only a general one regardless of the tree species tested, but also comparable to that of a solanaceous species, *Solanum viarum*.

P-1154 In Vitro Morphogenesis from Cotyledon Derived Nodular Callus of Pigeon pea

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For the effective use of tissue culture in crop improvement it is essential to devise methodology for repeatable regeneration. Regeneration through callus cultures of pigeon pea (Cajanus cajan (L) Millsp.) has been generally difficult and there are no adequate reports on morphogenesis. In the present study it was possible to accomplish the induction of nodular callus from the cotyledons and to study the morphogenetic process leading to the formation of plants anatomically. Proliferation of cells was always initiated close to the cut end at the base of the cotyledon after four days of culturing on L6 media supplemented with 6-Benzyl aminopurine (0.1 - 2.25 mgl-1) in all the four genotypes studied (Icpl-227, Icpl-239, C-11 and PI-397039). Th frequency of callusing explants that gave rise to plants varied from 4-40%, the intermediate range of BAP concentrations (0.5 and 1.0 mgl-1) and the genotype Icpl-227, gave a high frequency of regeneration. Although the developmental pathway observed is not overtly identical to zygotic embryogeny, young regenerated plants resembled zygotic seedlings both morphologically and anatomically. The results obtained in the present study is unique in that, it shows regeneration of pigeon pea plants on a single medium as one step process, besides tracing the morphogenetic pathway anatomically.

P-1155 Somatic Embryogenesis and Organogenesis in the Himalayan Yellow Poppy Meconopsis paniculata.

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Meconopsis paniculata (D.Don) Prain is a threatened taxon of potential horticultural value and is restricted to higher altitudes of Himalaya. Subculturable calli could be induced from hypocotyl segments from 3-month-old seedlings on Murashige and Skoog's (MS) medium supplemented with 10^{-6} M Kinetin (Kn) + 10^{-5} M α-naphthalene acetic acid (NAA). The suspension cultures were initiated from the calli in a similar medium but with 2,4-dichlorophenoxy acetic acid (2,4-D). Somatic embryos with normal bipolar organisation differentiated in 3-4 weeks from subculture but failed to develop into plantlets. In subculture of the calli on agarified MS medium supplemented with various auxins and cytokinins shoots differentiated. The regenerated shoots were induced to root in auxin treatments. Our result suggests that in M. paniculata ex situ conservation through organogenesis to derive plantlets in vitro is a better proposition than aiming to induce plantlets through somatic embryogenesis.

P-1156

A Two Dimensional Electrophoretic Analysis of Protein during Somatic Embryo formation in Cell Suspension Cultures of Oryza. K. OZAWA[1], D.H. Ling[2] and A. Komamine[3]. [1] Depertement of Cell Biology, National Institute of Agrobiological Resources, Ibaraki, Japan 305. [2] South China Institute of Botany, Academia Sinica, Guangzhou, China. [3] Biological Institute, Faculty of Science, Tohoku University, Miyagi, Japan, 980.

Little progress has been made in the physiologica and biochemical investigation of embryogenesis in suspension cultures of gramineae, because of the difficulty in obtaining a system in which embryogenesis occurs at high frequency from small cell clusters. A finely dispersed embryonic cell suspension cultures were obtained from the embryonic callus initiated from young panicles of a interspecific hybrid F1 (Oryza sativa x Oryza latifolia). A system in which somatic embryogenesis occurred at high frequency(about 60%) from small cell clusters (approximately 60-200 μm) was established using this cell suspension cultures. Using two-dimensional gel electrophoresis, total soluble protein and protein labeled in vivo with [35S]-methionine were extracted from regenerating and nonregenerating cultures and the gelsand autoradiographs analyzed to identify different sets of proteins associated with development in vitro. specific polypeptide (49kD) related to embryo genesis was identified.

P-1157 Embryogenesis specific protein changes in birch (Betula pendula Roth.) in vitro cultures.

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Two cell lines of birch, one potentially embryogenic, given the right inductive conditions, and one which never has shown any embryogenic capacity, were both given conditions inductive and non-inductive for somatic embryogenesis. The differences between inductive and non-inductive conditions were the content of 2,4-D, Kinetin, and casein hydrolysate, which was reduced to 1/20 under inductive conditions. Cells from these treatments were harvested on 104 μm sieves, washed with 0.5 M NaCl + 0.05 M CDTA to wash off proteins loosely attached to the cell wall. The washings were dialysed, frozen in liquid N_2 and freeze-dried. The remaining cells were either frozen whole, or the cell walls were isolated. Proteins were solubilized in SDS and mercaptoethanol. The extracted proteins from the supernatant were separated by one-dimentional SDS-polyacrylamide gel electrophoresis on BIORAD minigel systems. Both 8 and 16% gels were run in all cases. The gels were silver stained. Proteins specific for embryogenic cultures under inductive conditions were found in samples from whole cells, whereas, in the samples from isolated walls and "cell washings", certain proteins seem to disappear when the cells enter the embryogenic state. Proteins from the different samples which are seen to alter (appear or disappear) have been isolated as bands from gels, electroeluted and injected to produce antibodies (both polyclonal and monoclonal). These antibodies will be used for immunolocalisation studies together with characterization of the specific proteins. In the case of the proteins which disappear antibodies will be used to determine whether the proteins really vanish or simply become unextractable.

P-1158 First Enzyme of the Shikimate Pathway Enhacement with the Differentiation of Xylem in Tobacco Callus. J.E. PINTO, Jian-Min Zhao,K.M. Herrmann. Laboratory of Tissue Culture, ESAL, Lavras-MG, 37200, Brazil (JEP) and Department of Biochemistry, Purdue University, West Lafayette, IN 47907(JZ, KMH).

The activity of 3-Deoxy-D-arabino-Heptulosonate 7-phosphate (DAHP) synthase, the first enzyme of the shikimate pathway, was increased during xylogenesis in tobacco callus and potato plants. This increase of DAHP synthase activity correlate with the increase of staining in the tracheary element by antivodies against DAHP synthase from potato tuber. Transgenic plants with GUS fused to potato DAHP synthase promotor and its first 111 amino acid residues at the N-terminus, was induced to form new vascular structures by wounding. The activity of GUS was observed in the newly formed vascular regions.

P-1159

WITHDRAWN

P-1160 Expression of rol genes and insurgence of genetic tumours in Nicotiana glauca x Nicotiana langsdorfii hybrids. D. MARIOTTI*, M. Cardarelli°, P. Bogani§, M. Buiatti§ and P. Costantino°. *IREV-CNR, Roma; °CAN-CNR, Roma; §Ist.Genet., Univ. Firenze; DBGM, Univ. Roma, ITALY.

Agrobacterium rhizogenes rol B, C, and D genes have been individually introduced in N. glauca and N. langsdorfii plants. rol B-transformed plants of both species exhibit typical rol B-associated developmental and morphological characteristics (altered leaf morphology, increased formation of adventitious roots, etc.). N. glauca plants containing rol C show reduced apical dominance and a "bushy" appearance, also typical of rol C-transformed plants of other species. On the contrary, N. langsdorfii-rol C (and, to a lesser extent, rol D) plants generate spontaneous teratomatous tumours morphologically indistinguishable from the genetic tumours generated by N. glauca x N. langsdorfii hybrids. Moreover, a mutant hybrid incapable of generating genetic tumours could be made tumorous upon insertion of rol C. In view of the presence of endogenous rol genes in N. glauca and their absence in the N. langsdorfii genome we are currently testing the possible correlation between expression of endogenous rol C and D genes in hybrids plants and the insurgence of genetic tumours in these latter.

P-1161

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P-1162

WITHDRAWN

P-1163 Effect of Culture Filtrate of Fusarium udum on in vitro induced embryos from cotyledols of C.cajan genotypes.

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Somatic embryogenesis was achieved using cotyledonary explants in a no. of genotypes. Formation of somatic embryos was dependent on concentration of specific cytokinins and mineral nutrient formulations. On a high cytokinin medium, the distal halves of the isolated cotyledons expanded rapidly, turned green and induction of embryogenesis occurred. Reduction of cytokinins favoured maturation of the embryoids. Complete withdrawal of the cytokinins with introduction of small amount of auxin proved effective in germination of the somatic embryos.

This important crop suffers from wilt disease caused by a fungus Fusarium udum. Since in the present protocol, a high degree of embryogenesis is obtained, it was further used for achieving a resistant clone by challenging the cells with culture filtrate of Fusarium udum. The effect of culture filtrate at various volume percentages was tested at each stage of embryo development viz. induction, maturation and germination of the embryos. The frequency of embryo development was calculated for five genotypes of C. cajan.

P-1164 Field Evaluation of Herbicide-Tolerant Hybrid Poplar Somaclones. C. H. MICHLER, T. M. Voelker, and R. J. Moioffer. USDA Forest Service, North Central Forest Experiment Station, Forestry Sciences Laboratory, Rhinelander, WI 54501.

Field evaluation of in vitro selected, herbicide-tolerant hybrid poplars is necessary to determine productivity traits of modified clones. In initial tests (Year 1), two glyphosate-tolerant and four sulfometuron methyl-tolerant somaclones were challenged with herbicide in replicated field trials in comparison tests to their unmodified, parent clones. Glyphosate-tolerant trees were treated with 10.5, 15.75, and 21.0 mmol/l Roundup; sulfometuron methyl-tolerant trees with 0.69, 1.37, and 2.06 mmol/l Oust. Stem and foliar symptoms, and tree height were recorded weekly; branch number and length, and trunk diameter were determined at the end of the growing season. Unmodified parent clones had greater mean height increase than herbicide-tolerant clones in unchallenged plots. But, with herbicide treatment, there was no significant difference in tree height. All herbicide-tolerant somaclones had increased branch number compared to parent clones. Glyphosate-tolerant somaclones achieved the greatest field tolerance. Most notably, NC-13288 survived treatment at 21.0 mmol/l while the parent clone was killed at 10.5 mmol/l. The best sulfometuron methyl-tolerant somaclone, NC-13286 had no apparent damage when treated at 1.37 mmol/l, but the parent clone, NC-11390 was killed at 0.69 mmol/l. Furthermore, all commercially important, herbicide-tolerant somaclones lacked herbicide-induced foliar damage, but had similar terminal growth damage that was observed on parent clones.

T-1001 The Effects of Oxidant Stress on $[Ca^{2+}]_i$ Regulation in Mouse Epidermal JB6 Cells. P.T. JAIN, I.K. Berezesky and B.F. Trump. Univ. of Maryland Sch. of Med., Dept. of Pathology and MIEMSS, Baltimore, MD 21201.

Oxidant stress represents an important mediator of acute and chronic cell injury following inflammation, xenobiotics and tumor promoters. Changes in $[Ca^{2+}]_i$ represent potentially important mediators in the transmembrane signalling involved in immediate and early gene expression, terminal differentiation, cell division, and cell toxicity. JB6 cells (Clone 41 promotable; Clone 30 non-promotable) were cultured in DMEM medium containing 10% FCS. Cells were loaded with Fura-2/AM for 60 min at 25°C in CMSS and studied using digital imaging fluorescence microscopy for imaging and quantitation of $[Ca^{2+}]_i$. Cell viability was assessed by trypan blue or propidium iodide. Xanthine (X) ranged in concentration from 10^{-6} to 10^{-3} M; xanthine oxidase (XOD) was 50 μU-50 mU/ml. X/XOD treatment was progressively toxic to both Clone 30 and Clone 41 at concentrations from 10^{-4} to 10^{-3} M. The toxicity was 4-fold greater for Clone 30 than for Clone 41. $[Ca^{2+}]_i$, normally at ~100 nM, increased rapidly following exposure to X/XOD. In most cells, the increase was uniform in the cytosol; in some cases, there was evidence of increased Ca^{2+} in the nuclei. The initial increase of $[Ca^{2+}]_i$ due to X/XOD was observed at the same time for both clones. However at ≥ 1 mM xanthine, the rate of increase of $[Ca^{2+}]_i$ was moderately faster in Clone 41 compared to Clone 30, and the highest level of $[Ca^{2+}]_i$ was significantly greater and sustained for 20 min longer in Clone 30. The longer time period of higher sustained $[Ca^{2+}]_i$ in Clone 30 may explain its higher toxicity, and may result in differential growth stimulation in Clone 41. (Supported by JHU/CAAT.)

T-1002 Development and Application of a Human Cell Line Expressing 5 cDNAs Encoding Xenobiotic-Metabolizing Enzymes. C.L. CRESPI, B.W. Penman, F.J. Gonzalez, H.V. Gelboin and R. Langenbach. Gentest Corporation, Woburn, MA, 01801; NIH/NCI, Bethesda, MD 20816; NIH/NIEHS, Research Triangle Park, NC 27709.

Considerable progress has been made in the development of mammalian cell lines which stably express cDNAs encoding cytochromes P450 (CYP) and other xenobiotic metabolizing enzymes. These cell lines have the potential to reduce reliance on primary cells or tissue extracts to accomplish metabolic conversions. Utilization of these cell lines for screening applications has been limited because most cell lines express a single cDNA while multiple enzymes are often involved in chemical toxicity. Using two plasmid vectors, we have developed a human B-lymphoblastoid cell line which stably expresses 5 cDNAs encoding human CYP1A2, CYP2A6, CYP2E1, CYP3A4 and microsomal epoxide hydrolase in addition to native CYP1A1 activity. Stability of cDNA expression was established using a panel of cytochrome P450 form-specific enzyme assays. The new cell line, designated MCL-5, was utilized for testing the mutagenicity of benzo(a)pyrene, 3-methylcholanthrene, aflatoxin B_1, N-nitrosodimethylamine and N-nitrosodiethylamine. Significant induction of mutation was observed after exposure to ng/ml concentrations of these chemicals. MCL-5 cells were >1000 fold more sensitive to these carcinogens than the parent (unmodified) cell line. The MCL-5 cell line shows considerable promise as a screening system for potential human carcinogens.

T-1003 Studies on the Efficacy of Chemopreventive Agents with the Bleomycin Assay. Z. TRIZNA and T.C.Hsu. Depts. of Cell Biology and Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas

The protective effects of chemopreventive agents were studied on mutagen-induced chromosomal breakage using human lymphoblastoid cell lines and freshly cultured lymphocytes from head and neck cancer patients and healthy controls. Ascorbic acid (AA), n-acetyl-l-cysteine (NAC), alpha-tocopherol-acid succinate (TAS), and 13-cis-retinoic acid (CRA) were tested. Mutagen-sensitivity was determined using the bleomycin assay. AA, NAC, TAS and CRA diminished mutagen-induced chromatid breakage in a dose-dependent manner in lymphoblastoid cell lines. The range of the decrease of chromosomal breakage in lymphocytes was the following (with dose ranges in parentheses). AA: 21-58% (10^{-5}-10^{-3}M, NAC: 23-73% (10^{-4}-10^{-2}M), TAS: 18-42% (10^{-8}-10^{-2}M), CRA: 3-39% (10^{-8}-10^{-5}M). The results demonstrated a dose-dependent protective effect mediated in vitro by AA, NAC, TAS, and CRA against mutagen-induced chromosomal damage. These concentrations are comparable to those achieved in different clinical applications or in human dietary studies. A similar phenomenon in vivo may explain the differences in occurrence of head and neck cancer between populations with different dietary backgrounds. The protection against genetic toxicity may be an important part of the chemopreventive effects of the compounds tested. The bleomycin assay may be used for studying compounds with presumed chemopreventive properties. The test may be applied to follow the course of clinical chemoprevention trials.

T-1004 Cultured Mesangial Cells in the Study of Cadmium Nephrotoxicity. T.A. CHIN and D.M. Templeton, Department of Clinical Biochemistry, University of Toronto, Toronto, Canada M5G 1L5

Cadmium is a toxic metal that has been shown to affect the renal function of a significant portion of the general population (Buchet et al., Lancet 336:669 (1990)). Glomerular dysfunction is an important component of Cd-induced nephropathy, and we have found that the mesangial cell (MC) is a target for cytotoxity in the isolated glomerulus. To examine the potential role of mesangial cytotoxicity in Cd-induced renal dysfunction, we studied the effects of low concentrations of $CdCl_2$ on several biochemical parameters in cultured rat MC. MC cultures were established from isolated rat glomeruli as previously described. Cultures were maintained in RPMI 1640 medium with 10% calf serum and characterized by their morphology, histochemistry and contractile response to angiotensin II. At 0.5 μM, Cd^{2+} caused a 4-fold elevation in intracellular glutathione over 8 to 24 h, and was a potent inducer of the sulfhydryl-rich protein metallothionein (MT). Cd^{2+} inhibited DNA synthesis in these cells with an EC_{50} of 5.4 ± 0.4 μM and prevented sulfation of matrix proteoglycans at ca. 10 μM. Depletion of glutathione with buthionine sulfoximine decreased this value to 1.8 ± 1.0 μM, while pre-induction of MT with Zn^{2+} was protective, raising the EC_{50} to 17.6 ± 0.7 μM Cd^{2+}. Only at concentrations of 20 μM Cd^{2+} and higher were significant effects on cell viability, attachment and protein synthesis observed. Depolymeriztion of the actin cytoskeleton, which is expected to impair the functional contraction of MC, was caused by Cd^{2+} at concentrations well below the EC_{50} for protein synthesis. Cd-MT complexes have been reported to be more toxic than Cd^{2+} to renal epithelial cells in culture. However, although ^{109}Cd was taken up by MC with similar kinetics from both Cd-MT and $CdCl_2$, Cd-MT did not affect DNA synthesis, actin structure or MC viability at Cd concentrations up to 60 μM. We conclude that ionic Cd^{2+} is the most toxic form of this metal to mesangial cells. While these cells respond to sub-μM concentrations of Cd^{2+} by increasing their levels of glutathione and metallothionein, presumably protective responses, only slightly higher levels may impair the regenerative capacity of mesangial cells, in addition to interfering with their specialized functions of contraction and matrix synthesis.

T-1005 In Vitro Differentiation of Hamster Pulmonary Neuroendocrine Cells. M. EMURA[1], A. Ochiai[1], M. Riebe-Imre[1], B. Panning[1], I. Paulini[1], U. Mohr[1], and D.L. Dungworth[2]. [1]Med. Hochschule Hannover, 3000 Hannover 61, FRG; [2]University of California, Davis, USA.

Bronchopulmonary neuroendocrine cells have long been regarded as a source of small cell lung cancers and quite recently their secretory products have become closely associated with the contraction/relaxation of airway smooth muscles. In spite of such importance, exact knowledge about the function, biology and origin of this cell type has scarcely been established. The current paper describes induction by hypoxia of a phenotype evidently representing that of in vitro pulmonary neuroendocrine cells in a cloned fetal Syrian hamster lung epithelial cell line. This cell line usually grows undifferentiated under conventional conditions. In an oxygen concentration of as low as 5%, the cell growth retarded from 11 to 25h of population doubling within a few days. The cells became polygonal with thick cytoplasm and round nuclei containing homogeneous chromatin. With a cultivation time of several weeks under hypoxia, cells started to secrete serotonin and simultaneously demonstrated marked Grimelius staining and immunohistochemical reaction of bombesin and chromogranin A. Calcitonin was detectable in a small fraction of cells. Collagen gel as a substratum in medium intensified not only the serotonin secretion more than 2-fold (14 nM), but also the above immunohistochemical reactions.

T-1006

Effects of Exposure to Polychlorinated Biphenyls (PCBs) on Natural Cytotoxicity of Earthworm Coelomocytes. M. Suzuki[1], E.L. COOPER[1], G.S. Eyambe[2], A.J. Goven[2], L.C. Fitzpatrick[2], and B.J. Venables[2,3] [1]Department of Anatomy and Cell Biology, School of Medicine, University of California Los Angeles, Los Angeles, CA 90024, [2]Environmental Effects Research Group, Department of Biological Sciences, University of North Texas, Denton, TX 76203 and [3]TRAC Laboratories, 113 Cedar St, Denton, TX 76201.

Mammals possess spontaneous cytotoxic cells, which have been studied extensively. Human and murine peripheral blood cells include a small percentage (3%) of "natural killer" (NK) cells. NK cells exhibit significant in vitro cytotoxicity against a variety of allogeneic and xenogeneic target cell lines. Natural killer cells are involved in immunologic surveillance against cancer. The possibility that some earthworm coelomocytes have natural killer function similar to that seen in mammals has been little explored. Coelomocytes of the earthworms *Eisenia fetida* and *Lumbricus terrestris* demonstrated significant spontaneous cytotoxicity against allogeneic and xenogeneic target cells. In a 24 hr trypan blue assay, allogeneic killing by coelomocytes from distinct *Lumbricus* individuals was significant at a p value of 0.04, while that between *Eisenia* individuals had significance of $p < 0.002$. Cytotoxicity between the two species was significant at a level of $p < 0.0005$. Polychlorinated biphenyls (PCBs) are artificial compounds which have been discarded with other man-made waste into some earthworm habitats and which resist degradation. Allogeneic cytotoxicity assays using cells from *Lumbricus* exposed to PCBs were inconclusive, but suggest that PCBs have an effect on natural killing. This in vitro technique may be a powerful way to further explore the mechanism of cell recognition and immunotoxicity. Supported in part by research grants ES04811 from NIH and R818642 from EPA.

T-1007 In Vitro Effect of Toxicants Derived from Polluted Sediments on Activities of Hemocytes from the Oyster, Crassostrea virginica. F.-L. E. CHU, A. Volety and R. Hale. Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Chemiluminescence (CL) and chemotactic responses of oyster hemocytes exposed to a mixture of toxicants extracted from sediments of the heavily polluted Elizabeth River, Virginia were assessed. Oyster hemocytes were incubated with different dilutions of the toxic mixture (100, 50, and 25% aqueous toxic extracts) at 15 ± 1°C. The undiluted exposure mixture consisted of over 100 individual compounds at a total concentration approximately 2.9 mg/l (ppm). The dominant compound present was naphthalene which contributed over 30% of the total observed in the extract. Other major aromatics observed included phenanthrene, methylnaphthalenes, acenaphthene fluorene and carbozole. Chemiluminescence of hemocytes was measured at 0, 0.5, 1.0, 1.5 and 2.5 hrs after incubation at 15 ± 1°C. Chemotaxis of hemocytes was determined at 2.0 and 3.0 hrs at 20 ± 1°C. Results of three trials indicated the following: At 0 to 1.0 hrs, the highest CL was recorded for hemocytes exposed to 0% toxic extract and declined with each increase in toxic extract concentration. However, CL responses measured at 2.5 hrs appeared to be higher in the hemocytes incubated in 100, 50, and 25% toxic extracts compared to the control (0% aqueous toxic extract). Similarly, chemotactic activity determined at 2.0 and 3.0 hrs showed enhanced zymosan stimulated chemotactic response in hemocytes exposed to toxic extracts. The hemocytes exposed to the 50% toxic extract had the highest percentages of chemotaxis. Aqueous extracts derived from contaminated sediments apparently modulate the CL and chemotactic responses in oyster hemocytes and these responses changed with time. The mechanisms responsible for the CL and chemotactic stimulation in hemocytes after in vitro exposure to toxic extracts longer than 1.5 hr are not known and need to be investigated.

T-1008

A Colorimetric Assay for Assessment of Drug Sensitivities in African Trypanosomes.

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Resistance of pathogenic African trypanosomes (protozoan parasites) to the few available drugs poses an increasing problem for chemotherapeutic control of human and livestock trypanosomiasis. Improved control strategies require new antitrypanosomal drugs or tests which enable surveillance of the status of drug sensitivities of trypanosome populations.

A colorimetric drug sensitivity assay initially developed for cancer cells (Skehan *et al.*, 1990, J. National Cancer Institute 82, 1107-1112) was investigated for its suitability to assess drug susceptibilities in *Trypanosoma congolense*. Bloodstream trypomastigotes were propagated axenically at 34°C and a 4.5% CO_2 atmosphere. The colorimetric drug sensitivity assay gave reliable results when trypanosomes from logarithmic growth phase were seeded at a density of 1×10^5 to 1×10^6 organisms/ml and were incubated with the drug for 48 hours without medium change. The endpoint of the assay was determined by staining fixed trypanosome-protein with sulforhodamine B. The optical density measured at 540 nm increased in direct proportion to the number of trypanosomes in the range of 5.0×10^4 to 8.0×10^5 organisms/200µl sample. The assay can be used for screening antitrypanosomal compounds. Furthermore, the assay was effective in showing differences in susceptibilities of drug-sensitive and drug-resistant *T. congolense* populations.

T-1009 Cytotoxic Effects of FK506 on Cultured Human Renal Proximal Tubule Cells. M. ATCHERSON, K. Baghelai and A. Trifillis. DMRT and Department of Pathology, School of Medicine, University of Maryland, Baltimore, MD 21201.

FK506 has been used as the primary immunosuppressive agent after a variety of organ transplants, and has been reported to have up to 100 times the immunosuppressive effect of cyclosporine with less nephrotoxic effect. The cytotoxic effect of FK506 on cultures of normal human renal proximal tubule cells (HRPTC) was measured by neutral red (NR) inclusion and trypan blue (TB) exclusion. Cultures of HRPTC from the same donor were exposed to 0, 20, or 40 μg/mL FK506 in a vehicle of 40% w/v HCO-60 castor oil in absolute ethanol, and NR inclusion was determined on days 1-7 and 10-12. Viability of HRPTC exposed to 40 μg/mL FK506 was also measured by TB exclusion on day 7. Twenty μg/mL FK506 caused a transient decrease in NR inclusion to 79% (±11%, 1 SD) of the vehicle control (expressed as 100%) on day 5 (n=3); NR inclusion increased to 106% (±9%) on day 7 and to 110% (±3%) on day 12. Forty μg/mL FK506 decreased NR inclusion to 61% (±8%) of the vehicle control on day 7 (n=3), with recovery to 86% (±5%) on day 12. TB exclusion decreased to 67% (±10%) of the vehicle control after treatment with 40 μg/mL FK506 for 7 days. Thus treatment with 40 μg/mL FK506 for 7 days exhibited similar degrees of cytotoxicity when measured by both NR inclusion and TB exclusion. These data indicate that in vitro treatment of HRPTC with 20-40 μg/mL FK506 for 5-7 days caused reversible damage as evidenced by increased NR inclusion at day 10. However, treatment with 200 μg/mL of FK506 for 4 days decreased NR inclusion to less than 10% of the vehicle control. These findings of transient cytotoxicity with very high doses of FK506 (ie. 20,000 times the therapeutic plasma level of 2 ng/mL) are consistent with the clinical findings of FK506-induced short-term nephrotoxicity which is followed by recovery. (Supported by a grant from the Maryland Kidney Foundation)

T-1010 Human Corneal Epithelial Primary Cultures and Immortalized Cell Lines: In Vitro Model for Ocular Studies. C. R. KAHN*, I. H. Lee and J. S. Rhim. Gillette Med. Eval. Labs*, Gaithersburg, MD 20879, and National Cancer Institute,Bethesda, MD 20892.

In order to model the external ocular surface in vitro, we have established immortalized human corneal epithelial cell (IHCEC) lines using Ad12-SV40 hybrid virus or plasmids containing the SV40 early region genes. Primary cultures were established in low calcium, serum-free medium, passaged once and either infected or transfected with pRSV-T or pSV_3neo. Control, primary cultures senesced after 5 passages. Only IHCEC lines that were not shedding virus were further characterized. Primary cultures, at confluence ($5x10^4$ cells/cm^2), exhibited cobblestone morphology. IHCEC lines had saturation densities ranging from3 to $6.8x10^4$ cells/cm^2 and also exhibited cobblestone morphology. Mean diameters of the primaries (16.6uM +/_ 0.3) and IHCEC lines (16.2uM +/- 0.7) were determined by Coulter analysis. Population doubling times (DT) of the primary cultures were 24.4 +/- 0.7 hrs, compared to 19.5-36.5 hours for IHCEC lines. Growth of cells on collagen membranes at air-liquid interfaces yielded tightly packed surfaces similar to those seen in vivo. Immunofluorescence revealed the synthesis of corneal specific cytokeratins and large T antigen by IHCEC. Isozyme analysis confirmed the human origin of the cells and karyotype analysis showed them to be hyperdiploid. Two of the 7 lines cloned in agar formed colonies. Keratinocyte (epithelial) lines from 13 individual corneal donors have been established as well as one keratocye (fibroblast) line selected for growth in low calcium medium.

T-1011 Cytotoxicity Evaluation from the Viewpoint of Cell Recovery. K.IMAI and M.NAKAMURA, Dept. of Biomaterials,Osaka Dental Univ., Otemae, Chuo-ku, Osaka 540, JAPAN

Cytotoxicity of biomaterials from the viewpoint of cell recovery was examined. First, cell recovery of Gin-1 cells following 24 hours treatment with various substances was studied. Conventional cytotoxicity test based on cell growth rate were performed on the same substances. Metal ions (Ag, Cu, Pd, Ti) and plastic monomers for dental use showed cytotoxicity at a certain level with both test, while PBS(-) tested appeared to be cytotoxic due to no cell growth with the conventional cytotoxicity test, compared to non cytotoxic after a recovery phase of 4 days. A discrepancy between the two test methods seems to show a necessity of developing a cytotoxicity test which considers aspects in addition to the conventional one, such as a cell recovery test. Second, a cell recovery test was carried out with 7 kinds of dental adhesive monomers. The degree of cytotoxicity varied depending on the kind of hydrophobic group. From the present findings, it became apparent that metabolic stoppage should be considered separately from cell death. Expression of sub-lethal damage as well as delayed cytotoxicity are approachable with the cell recovery test. Therefore, we can discover a wider range of aspects of materials' effects on cells with this test.

T-1012 In Vitro Cytotoxicity Testing: Biological and Statistical Significance F.A. BARILE, S. Arjun, D. Hopkinson. Department of Natural Sciences, City University of New York at York College, Jamaica, NY 11451.

This study was designed to develop an in vitro model for predicting acute human and animal toxicity. Rat lung epithelial cells (L2) were tested for their ability to incorporate radiolabeled amino acids into newly synthesized proteins, in the absence or presence of increasing doses of the test chemical. The MTT assay and growth studies were also performed as parallel measures of toxicity. IC10, IC50 and IC75 values (10%, 50% and 75% inhibitory concentrations, respectively) were extrapolated from dose-response curves after linear regression analysis. The biological significance of the results of testing 50 chemicals shows that the experimental IC50 values were more accurate predictors of human toxicity than equivalent toxic blood concentrations derived from rodent LD50s. IC50s from 24-hr MTT and 72-hr growth studies were similar to results of 24-hr protein synthesis experiments. The project also introduces hypothesis testing for linearity as an important statistical parameter for evaluating the significance of dose-response curves, and which has not been routinely included in similar studies. These procedures may supplement or replace current animal protocols for human risk assessment. (Supported by NIGMS and NHLBI GM08153).

T-1013 Neutral Red (NR) Assay for Potency Study of Chemicals in Common Use. E. BORENFREUND and H. Babich. The Rockefeller University, New York, NY 10021

Human cells, i.e., keratinocytes (NHEK), fibroblasts (HFF), endothelial (ENDO), melanoma (SK-Mel/27), and hepatoma (HepG2) cells were used as indicators in the NR assay to evaluate the potencies of chemicals to which humans are commonly exposed. Nicotine was more cytotoxic than its metabolite, cotinine, which was more toxic than the related test agents, nicotinamide and nicotinic acid. Nicotine, but neither cotinine, nicotinamide, nor nicotinic acid, induced cytoplasmic vacuolization in all cell types. The toxicity to HepG2 cells of eugenol, a component of clove cigarettes and an inducer of oxidative stress, was pronounced in cells pretreated with glutathione depleting agents and with diethyldithiocarbamate, an inhibitor of superoxide dismutase. Eugenol toxicity was enhanced in the presence of hepatic S9 microsomal fractions. Caffeine and its metabolite, theophylline, showed comparable toxicities to all cells, except that the ENDO cells were 2-3 times more sensitive to caffeine. Phenylpropanolamine (PPA), found in diet pills, induced marked vacuolization in ENDO cells only. This could account for the increased uptake of NR, giving the appearance of reduced sensitivity as compared to HFF, HepG2, and SK-Mel/27 cells. PPA potentiated the cytotoxicity of caffeine to HepG2 cells.

Supported, in part, by Schering-Plough.

T-1014

A 3-Dimensional Human Skin Culture System Used In Toxicity Testing Of Topically Applied Test Agents. D. TRIGLIA, T. Donnelly, I. Kidd and S. Sherard Braa. Advanced Tissue Sciences, Inc. (formerly Marrow-Tech, Inc.); La Jolla, CA 92037.

A novel three-dimensional, human skin model, developed at Advanced Tissue Sciences, has been successfully used as a substrate for evaluating the toxicity of topically applied, undiluted or high concentrations of test agents similar to those used in human or animal skin patch tests. The Barrier Function Model consists of several layers of actively dividing, metabolically active, neonatal foreskin-derived fibroblasts grown on nylon mesh in the presence of ascorbate. On top of this dermis is a basal layer of epidermal keratinocytes, several layers of differentiated keratinocytes and stratum corneum. The tissue substrates were placed atop a Millicell polycarbonate culture insert (3 μm pore size) and serum-free DMEM-based medium was placed below. Test articles were applied undiluted (or diluted in medium) onto the stratum corneum and incubated for 1 hour to overnight; the mesh was assayed for cytotoxicity using MTT and the culture medium beneath the insert was assayed for release of PGE_2 (inflammatory mediator) and LDH (cell membrane integrity). We tested 15 petrochemicals, 7 hair conditioners and a panel of cosmetic products. Using Cooper's criteria, we have correctly classified 7/7 hair conditioners using the MTT assay compared with in vivo rabbit Draize unwashed eye scores, 14/15 petrochemicals using the LDH assay compared with in vivo FHSA primary dermal irritation data and 10/11 cosmetics using the PGE_2 assay compared with in vivo human skin irritation data.

T-1015 Comparison of the Results from 100 Different Cytotoxicity Assays of the First 10 MEIC Chemicals. B. EKWALL*, F. Barile, H. Bjerregaard, C. Chesne, R. Clothier, P. Dierickx, M. Ferro, G. Fiskesjö, L. Garza-Ocanas, M.J. Gómez-Lechón, M. Gülden, B. Isomaa, T. Jacobsen, U. Kristen, M. Kunimoto, S. Kärenlampi, L. Lewan, M. Nordin, G. Persoone, T. Sawyer, H. Seibert, R. Shrivastava, A. Stammati, M. Tingleff-Skaanild, D. Triglia, C. Tyson & E. Walum. (BE) Dept. Toxicology, Univ. of Uppsala, BMC, Box 594, S-75124 UPPSALA, SWEDEN

The Multicenter Evaluation of In Vitro Cytotoxicity (MEIC) is organized by the Scandinavian Society for Cell Toxicology. International laboratories with in vitro tests of toxicokinetics or general toxicity are invited to test 50 reference chemicals. The results will be evaluated for relevance to acute, chronic, target organ, skin, and eye toxicity with use of human data. At present, 79 laboratories are taking part with more than 200 in vitro tests. Preliminary evaluation of the results for 10 chemicals of 50 test methods indicated a good prediction of acute lethal as well as sublethal human toxicity, in parity with LD50 prediction (B. Ekwall et al. ATLA(1991)226-233).

The present study compares the cytotoxicity of the first 10 MEIC-chemicals measured in 100 different tests from 27 laboratories. Most methods used animal or human cell lines and organ-specific cells in primary culture. Most of these cytotoxicity tests measured inhibition of basal cell functions (cell counts, protein, NR, LDH, MTT, etc.). In tests with an exposure time of 24h or more, all such measurements gave approximately the same result. This must be due to a close integration of all basal cell functions/organelles, spreading various types of primary injury to the whole cell, in 24 hours. The close integration of cell functions will facilitate future basal cytotoxicity testing. Thus, one or a few tests may measure all types of basal cytotoxicity.

Some tests assayed organotypic functions. Inhibition of cell contractility at lower than basal cytotoxic concentrations predicted muscle-relaxant properties in man of some chemicals.

T-1016

A Cell Culture Model for Lung Toxicity. K.A. COSTA and J.M. Cerreta, College of Pharmacy Allied Health, St. John's University, Jamaica NY 11439.

The lung is a principal site of injury for many xenobiotics, including gases, organic dust, herbicides, drugs and metals. A major manifestation of lung toxicity is pulmonary fibrosis. Animal models of fibrosis indicate that changes in lung morphology are paralleled by alterations in lung connective tissue (CT) proteins (ie. elastin). In vitro pulmonary toxicity tests have been difficult to devise since lung morphologic assessment is not possible in cell culture. However, the rationale for the present study is that changes in CT proteins observed in animal lungs following challenge with toxicants are paralleled by similar changes in in vitro systems when challenged by the same agents. The ATCC cell line (CCL-216) used is derived from rat pleural mesothelium and is one of only 5 cell types known to produce insoluble elastin. Four known pulmonary toxicants were chosen: amiodarone, bleomycin, paraquat and $CdCl_2$. These agents have all been used to induce pulmonary fibrosis in animals. Cells were exposed for 24 hrs. to amiodarone, bleomycin, paraquat and $CdCl_2$ at the following levels: 10 ug/ml, 1 ug/ml, 2 ug/ml and 20 ng/ml, respectively. Following exposure, cultures were radiolabeled with ^{14}C-lysine. Elastin specific crosslinks desmosine/isodesmosine (des/isodes) were isolated using chromatography and electrophoresis. RNA was isolated by a guanidinium thiocyanate extraction. mRNA's were separated by electrophoresis. Following Northern blotting, elastin mRNA levels were measured by autoradiography. Data indicates elevated levels of des/isodes in cultures treated with the chosen toxicants. Preliminary data suggest that elastin mRNA levels are effected by these agents as well. This in vitro screen possesses the potential to detect the heralding events of fibrosis at two levels: mRNA transcription and protein synthesis. This screen could greatly reduce the number of animals employed in respiratory toxicity testing by serving as part of the early phase of product safety evaluation.

T-1017 An Automated Kinetic Microassay For Lactate Dehydrogenase Using A Microplate Reader. P. MCNEELA and P. Dehn. Biology, Canisius College, Buffalo, New York 14208.

Many parameters are utilized to detect structural or functional impairment of cells in vitro. Cytosolic enzyme leakage, e.g. lactate dehydrogenase (LDH), has been used as a sign of cytotoxicity. However for numerous samples it is time consuming. The development of a rapid, simple, quantitative, automated LDH microassay using a kinetic microplate reader will eliminate the need to use less sensitive and/or time-consuming tests and will allow for greater replication of sample sizes tested. Here we describe a kinetic microassay that measures LDH activity in both cells and culture medium. The microassay is a modification of that developed by Vassault and is dependent upon the oxidation of NADH when pyruvate is converted to lactate. The microassay was linear in the 10 to 300 U/L range with $y = 0.14231 X + 6.45237$ ($R^2 = 0.9914$) for 3 replicate runs. A time course study using 10, 150 and 300 U/L concentrations of LDH, from the linear portion of the curve, showed reaction rate linearity at each concentration for at least 2 min at 25°C. This microassay has application in measurement of the overall health of cells in culture, the estimation of cytotoxicity by the criterion of membrane damage, and in studies of organelles where enzyme markers must be analyzed. The automation increases the speed and reliability of the assay and will allow for larger sample sizes and hence more meaningful statistical analyses to be utilized. (Supported by NIH Tox (AHR-A) 1 R15 ES054444-01 to PFD and Arthur Vining Davis and Charles A. Dana Foundations undergraduate research training awards to PMcN.)

T-1018 Alterations in lectin binding to the plasma membrane of cultured epithelial tracheal cells after incubation in acidic buffer. M.L. BERTHEZENE, F. Rogerieux, M-A. Nahori, J. Bignon and C. Lambré. INSERM U 139, Hop. H. Mondor, 94010 Créteil, and INSERM 285 Institut Pasteur, Paris, France.

Exposure of human population to atmospheric acidity is associated with an increased incidence of pulmonary diseases such as hyperreactivity, and infection. Membrane glycoconjugates may represent a target for low pHs as: a) some glycosidic linkages are acid sensitive, b) sugars, especially sialic acids are key components of numerous receptors. Using plant lectins, membrane glycoconjugates of cultured epithelial cells from the guinea pig trachea were probed after incubation in buffers with acidic pH. Epithelial cells were isolated from the guinea pig trachea by protease digestion. They were cultured in medium 199 supplemented with 10% foetal calf serum at a seeding concentration of 5.10^5/ml. The medium was changed every two days and the cells were used after reaching confluency, usually five days after seeding. They were incubated for one hour at 37°C with either Vibrio cholerae sialidase as a positive control or in phosphate buffers with pH ranging from 7.3 to 5.5. After inhibition of the endogenous peroxidase activity with H2O2 1% in 70° ethanol, the cells were incubated with digoxigenin-linked lectin then with peroxidase-labelled antidigoxigenin Fab fragments. Lectins were from Arachis hypogaea (PNA) that is specific for the gal-ß 1-3 gal-Nac sequence, Sambucus nigra (SNA) and Maackia amurensis (MAA) that are specific for α 2-6 and α 2-3 linked sialic acids, respectively, and Galanthus nivalis (GNA) that is mannose-specific.

As expected, sialidase-induced desialylation lead to increased fixation of GNA and PNA, and to decreased fixation of SNA and MAA. After incubation in acidic pH from 7.3 down to 5.5, the vitality decreased from 90 to 75%. The fixation of the two sialic acid specific lectins diminished not significantly while fixation of PNA increased moderately; the most significant alteration was a large increase in GNA fixation. These results suggest a modification in the membrane expression of sialic acids rather than desialylation. Chromatographic analysis are in progress in order to evidence a possible release of sialic acids from the cell membrane following incubation in acidic pHs. Sialidase-induced membrane desialylation leads to the fixation of autoantibodies, complement activation and increased bacterial adherence. Through altering membrane sialic acids, exposure to acidic pH may have the same consequences as well.

T-1019 Testosterone-Induced Ultrastructural Changes in Primary Neonatal Rat Myocardial Cell Cultures. R. Melchert, T. Herron, and A. Welder. College of Pharmacy, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Anabolic-androgenic steroids have recently been shown to have unfavorable effects on the myocardium. *In vivo* administration of methandrostenolone to rats induced ultrastructural changes in the myocardium whereas *in vitro* administration of testosterone cypionate (TC) to primary neonatal myocardial cells in culture induced gross cellular injury. The purpose of this investigation was to examine *in vitro* ultrastructural changes in primary neonatal rat myocardial cell cultures exposed to TC. Evaluation of drug effects were made in cultures obtained from 3- to 5-day-old Sprague-Dawley rats. After the cells had been in culture for four days, they were exposed to 1×10^{-6} and 1×10^{-4} M TC for 1, 4, and 24 hr. Transmission electron microscopy (TEM), phase-contrast microscopy, and lactate dehydrogenase (LDH) release were used to evaluate cellular injury. TEM revealed a decrease in mitochondrial number as well as disruption of mitochondrial and plasma membranes in cultures exposed to 1×10^{-4} M TC at 24 hr. For this same treatment, phase-contrast microscopy revealed monolayer destruction while no effects were observed in cultures exposed to 1×10^{-6} M TC. Finally, significant LDH release occurred only in those cultures exposed to 1×10^{-4} M TC for 4 and 24 hr. These data suggest that acute administration of the anabolic-androgenic steroid testosterone cypionate to primary neonatal rat myocardial cell cultures induces gross morphological and ultrastuctural injury to the sarcolemma and mitochondria. (This work was supported by a NIDA FIRST award #DA05699).

T-1020 Induction of Tumor Necrosis Factor Activity in Laboratory Animals Treated with Hepatotoxicants. A. ROY, G.R. Soni, R. M. Kolhapure, K. Banerjee and P.S. Patki*. National Institute of Virology and *Department of Pharmacology, B. J. Medical College, Pune-411001, India.

In the absence of suitable animal model for hepatitis, we chose different hepatotoxicants to find their role in the induction of TNF in the serum. Adult Charles Foster rats, when fed orally with LD_{50} dose of CCl_4 released TNF in serum and the activity peaked in 24 hours. Swiss mice injected intra peritoneally with D-galactosamine also released TNFα but the activity totally disappered in 2 days. There is a definite correlationship between the TNF release and the liver damage. There is elevation of enzyme levels namely Alkaline phophatase and SGOT in the serum but no elevation of bilirubin level is observed in treated animals. Alpha Napthyl Isothiocyanate, a potent hepatotoxicant, neither releases TNF nor shows any sign of liver damage in rats when treated for three consecutive days.

T-1021 A Novel Analytical System to Monitor Cell Behavior in Response to Chemical Agents. C.R. KEESE and I. Giaever. Rensselaer Polytechnic Institute, Troy, NY 12180 and Applied Biophysics, Inc., 2080 Van Antwerp Road, Schenectady, NY.

We have developed an instrumental method to monitor the activities of mammalian cells in culture. ECIS (electric cell-substrate impedance sensor) detects changes in the electrical impedance of small gold electrodes used as substrata for cell growth. We have carried out extensive measurements with this approach and have documented its ability to quantify many complex in vitro cellular activities in a continuous fashion and with very high sensitivity. Activities monitored include cell attachment and spreading (1), cell growth, and cell motility (2). Since these are complex behaviors and require the cooperation of many cellular systems, it is not surprising that ECIS measurements are affected by a wide variety of chemical, biological, and physical agents. Characteristic responses of human fibroblasts, murine macrophages and bovine endothelial cells to several agents have been obtained with this method and will be discussed.

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(2) Giaever, I. and C.R. Keese, **Proc. Natl. Acad. Sci. USA, 88**, 7896 (1991).

T-1022 Cytotoxicity, DNA-binding and immunofluorescent localization of 3'-azido-2',3'-dideoxythymidine (AZT) in human, hamster and mouse cell lines. O.A. OLIVERO, F.A. Beland* and M.C. Poirier. Lab. of Cellular Carcinogenesis and Tumor Promotion, NCI, NIH, Bethesda, MD 20892. *Div. of Biochemical Toxicology, NCTR, Jefferson, AR 72079.

The thymidine nucleoside analogue AZT produces bone marrow and other toxicities in humans. In order to develop methods to assess the long-term toxicity of this drug, we have studied the incorporation of [^{3}H] AZT into the DNA of cultured cells, by radiolabeling and radioimmunoassay(RIA; with an anti-AZT antibody), and localization of the drug in chromosomes by immunohistochemistry. Semi-confluent human HL60, Chinese hamster ovary (CHO) and mouse NIH 3T3 cells were exposed to AZT (1 lM to 2 mM) and survival in culture was determined after two cell cycles. Cell survival observed with 0.8 mM AZT was 63.2% for HL60, 52.4% for CHO and 41.5% for NIH 3T3 cells. When cells were exposed to 0.8 mM [^{3}H]AZT for 24 hours, incorporation into DNA estimated by radioactivity was 0.82 pmol/lg DNA for HL60, 0.84 pmol/lg DNA for CHO and 4.49 pmol/lg for NIH 3T3 cells. By RIA the incorporation of AZT into the same DNA samples was 0.13 pmol/lg DNA for HL60, 2.80 pmol/lg DNA for CHO and 4.35 pmol/lg DNA for NIH 3T3 cells. Indirect immunofluorescence with the same anti-AZT antibody demonstrated a specific pattern of localization of AZT in metaphase chromosomes with higher concentrations of AZT in the telomeric regions of 19 of the 20 CHO cell chromosomes. These methods may be useful for assessing incorporation of AZT in a variety of tissues obtained in vivo.

T-1023 An In Vitro Screening System for the Nephrotoxicity of New Platinum Coordination Complexes using Rabbit Kidney Proximal Tubule Cells in Primary Cultures. H.TOUTAIN, F. Courjault, D. Leroy, I. Coquery and P. Mailliet. Unité de Néphrotoxicologie, Centre de Recherche de Vitry-Alfortville, Rhône-Poulenc Rorer, B.P. 14, 94403 Vitry sur Seine Cedex, FRANCE.

Platinum coordination complexes (PtCx) are potent agents against several types of neoplasms but nephrotoxic side effects are often associated with these drugs. In order to assess the nephrotoxic potential of new PtCx, primary cultures of rabbit proximal tubule cells (RPTC) were grown to confluency in antibiotic free, hormonally defined medium from calibrated proximal tubule fragments. 13 PtCx, including cisplatin (cDDP), transplatin (tDDP), carboplatin (CBDCA) and 10 new PtCx (9 spiro[(bridged bi or tricycloalkyl) ethylenediamine]platinum complexes and 1 carbamate platinum complex) were studied. The cytotoxicity of these PtCx, using the lactate dehydrogenase release method, and their effects at non-cytotoxic concentrations on functional parameters such as DNA and protein synthesis, glucose transport and Na-K-ATPase activity was assessed in 7 days RPTC cultures, after a 24 hour exposure.The cytotoxic potential was assessed by the $IC_{LDH}10$ which is the concentration required to cause a 10% increase in LDH leakage. A specificity parameter was calculated by $\Sigma(IC_{LDH}10/IC_x50)$, ($IC_x50$ is the drug concentration required to induce a 50% inhibition of each studied parameter (x)). tDDP is a non specific cytotoxic agent which is 3 times less cytotoxic than cDDP. CBDCA is 20 times less nephrotoxic than cDDP. These data show that in vitro nephrotoxic potential of cDDP, CBDCA and tDDP correlates with data reported in vivo (cDDP > > CBDCA > tDDP). Spiro[(bridged bicycloalkyl)ethylenediamine]PtCx nephrotoxic potentials are about 10 times lower than that of cDDP. Spiro[(bridged tricycloalkyl)ethylenediamine]PtCx exhibit variable cytotoxicity and always a low level of specificity, close to that of tDDP. The carbamate PtCx is 20% less cytotoxic than cDDP and shows a very low value of the specificity parameter. The in vitro nephrotoxicity of this PtCx is close to that of tDDP. This study demonstrates that RPTC primary cultures provide a good in vitro model for screening less nephrotoxic PtCx in relation to their chemical structures. This model makes it possible to discriminate between specific and non specific PtCx-induced nephrotoxicity.

T-1024 Potential Anti-leukemic Activity of Monomethyl Glycol Ethers Evaluated by in vitro Inhibition of Human (HL60) and Mouse (L1210) Leukemia Cell Culture Growth. J. A. GARNETT and M. P. Dieter, NIEHS, Res. Tri. Park, NC

Among 9 glycol ethers, ethylene glycol monomethyl ether (2ME) or the acetate (2MEA) at non-toxic doses markedly inhibited the progression of F344 rat mononuclear cell leukemia in an in vivo syngeneic tumor transplant model system (Dieter, et al. 1990). The efficacy of these anti-tumor agents were subsequently evaluated in vitro using HL60 and L1210 cell suspension cultures for up to 45 days. HL60 cells were maintained in suspension in RPMI + 10% fetal bovine sera with 1% pen/strep; L1210 cells were in Fischer's media with 10% horse sera. After 10d HL60 cells differentiated along a monocytic pathway, as shown by a positive myeloperoxidase reaction and a higher cytoplasmic to nuclear ratio, but there was no adherance or loss of proliferative capability. There was a maximum 86% reduction in HL60 cell numbers after 1 μM 2MEA at 16d, and a 95% reduction after addition of 10 μM 2ME. The reduction in cell numbers was consistent for up to 45d when media and chemicals were replaced every 2-3d. Discontinuation of either dosage regimen resulted in a rebound to control values. For L1210 cells, the maximum reduction in cell numbers by 1 μM 2MEA was 97% below control values at 23d; addition of 10 μM 2ME caused a 60% reduction in cell numbers. The efficacy of 2MEA as a potential anti-leukemic agent in vitro was 10-fold greater than that of 2ME in both human and mouse leukemia cell lines, possibly because of enhanced penetration of the cell membrane by the more soluble acetate ester.

T-1025 Risk Assessment of HIV/Infectious Disease in the Clinical and Research Laboratory: An Update.
S.L. SCHNEIDER and P. Lincoln Smith. The University of Texas Health Science, Department of Psychiatry, San Antonio, Texas and FDA, Bethesda Maryland.

Issues of HIV biosafety in the clinical and research laboratory have been of major scientific and political concern since 1985 with the isolation of retrovirus from all body fluids, and the CDC estimate that of the 1.5-3X10^6 infected U.S. people, 2.5X10^5 would have AIDS by 1991. The validity of CDC biosafety guidelines and the use of hepatitis virus (HBV) transmission as a model for HIV transmission has been strongly questioned as "not scientific". Recently, congress mandated that states must adopt CDC or equivalent guidelines; and the senate called for a bill requiring adherence to universal precautions against infectious disease transmission. OSHA standards have yet to be finalized for 1992. Despite the discrepancy between science and policy, working safely with HIV in the laboratory requires: 1) an understanding of the known risks of infection in relation to the handling of biological specimens (Class 2) compared to that of infected cell cultures and viral material (Class 3); 2) knowledge of guidelines for biohazard containment regarding handling of human specimens and infected cells; 3) consideration of issues relative to serologic screening of workers.

T-1026 In Vitro Neurotoxicity of Anti-AIDS Nucleoside Analogs. A.E. Weiss, M.J. PALMOSKI, O.P. Flint and F.B. Oleson. Investigative Toxicology, Bristol-Myers Squibb, Syracuse, NY 13221.

Clinical studies of anti-AIDS nucleoside analogs have implicated ddC, d4T and ddI as inducers of peripheral neuropathy, while AZT lacks any neuropathic effects. Since these neuropathies were not predicted by animal toxicology, the objective of this study was to compare the clinical potency with in vitro effects on micromass embryo neuronal cell cultures (J. Cell Sci. 61:247,1983). Cells obtained from 13 day rat embryo midbrains differentiated into neurons within 5 days of culture. Cytotoxicity was measured with the neutral red assay and the number of differentiated cell foci analyzed by hematoxylin staining and ATP levels. Results were expressed as the concentration of test agent inhibiting a parameter by 50% control values (IC50). When cells were exposed to test agent for 24 hours then cultured for a further 4 days in control medium the toxicity in vitro was similar to the relative in vivo toxicity. (n = 20-25)

	IC50 ± S.D. (μg/ml)		
Drug	Cytotoxicity	Foci	ATP
ddC	21.4±2.3	18.75±7.6	60.4±22.9
d4T	64.5±12.2	50.91±21.1	82.4±15.5
ddI	210.7±25.6	223.21±80.4	413.5±58.1
AZT	>1000	996.32±7.4	>1000

There was no significant difference between drug effects on cytotoxicity and differentiation. ATP analysis was generally a less sensitive indicator of toxicity than the histochemical techniques. The results indicate that this in vitro assay may have predictive value for assessing the neurotoxic potential of this class of antiviral agent.

T-1027 Vinblastine and Colchicine Cause Rapid and Pronounced Inhibition of Fast Axonal Transport Preceeding Neurite Degeneration in N1E.115 Neuroblastoma. D.J. BRAT and W.S. Brimijoin, Dept. of Pharmacology and Program in Molecular Neurosciences, Mayo Clinic/Foundation, Rochester, MN 55905

The role of axonal transport disturbances in toxic axonopathies has not been clearly defined by in vivo studies. We have used differentiated N1E.115 murine neuroblastoma cells as a model for examining neurotoxic phenomena in vitro. In serum-free media, 25-35% of N1E.115 cells extended neurites after 48 hrs. Vinblastine and colchicine, two drugs which disrupt microtubules and inhibit fast axonal trasport in vivo, decreased the percentage of cells extending neurites, with EC50 values of 33 ± 11 nM and 210 ± 110 nM, respectively. Vinblastine was even more potent in causing degeneration of previously extended neurites (EC50 = 1.1 ± 0.6 nM); colchicine was equi-effective in causing degeneration and inhibiting neurite outgrowth (EC50 = 360 ± 100 nM). Concentrations causing neurite degeneration were well below levels necessary for cytotoxicity measured by ^{51}Cr release (vinblastine EC50 = 26 ± 5 μM, cochicine EC50 > 1mM). Video enhanced contrast differential interference microscopy was used to determine the effects of these two drugs on fast axonal transport in vitro. Vinblastine (1 μM) almost completely inhibited bidirectional organelle motility in N1E.115 neurites within 20 min, several hours before degeneration occurred. Likewise, 1 μM colchicine reduced organellar motility to 33% of control values within 40 min. This rapid and pronounced inhibition of fast axonal transport, normally required to supply structural elements and metabolic enzymes to the periphery, may be the initial event leading to degeneration of established neurites.

T-1028 Gap Junctional Intercellular Communication and Screening of Potential Tumor Promoters. E. HONIKMAN-LEBAN and M.M.Shahin, Department of Chemical Protection and Photobiological Research in Vitro, L'Oreal Advanced Research Center, Aulnay-sous-Bois, 93600, France.

Alteration of cell to cell communication by chemicals may play a role in carcinogenesis. Inhibition of intercellular communication through gap-junctions (GJIC) will be used as the endpoint of a pre-screening assay for tumor promoters. On the other hand, the enhancement or restoration of GJIC may indicate an antitumor promoter potentiel. Different methodologies are available to quantify the GJIC : - diffusion of fluorescent dye (Lucifer yellow) after microinjection in one single cell or after scrape-loading of the treated cell cultures ; - recuperation of resistant colonies after establishment of metabolic cooperation between resistant and sensitive cells co-cultivated in a selective and treated medium. The effect of the tested chemical is then compared with the negative controls (dye, solvent, non treated cells) and the positive control as potent inhibitor of GJIC (12-o-tetradecanoyl-phorbol-13-acetate, TPA). In this regard, different compounds were tested for their susceptibility to act as tumor promoters. For example, phenobarbital, barbital, barbituric acid, nembutal were tested in the metabolic cooperation assay with the classical V79 protocol. Phenobarbital was also tested with rat liver cells in metabolic cooperation and dye transfer assays. With V79 cells (Chinese hamster lung fibroblasts), phenobarbital, barbital, nembutal showed a slight inhibition on GJIC. Phenobarbital gave a more important inhibitory effect with rat liver cells. This inhibition was not confirmed by dye transfer. Thus, these assay sytems gave us the opportunity to work on tissue specificity of tumor promoters. Therefore, it is important to establish the most adequate protocol (the most predictive, selective one). In this respect, under the initiative of the European Community, within the B.R.I.D.G.E. programme, seven research teams have formed a joint project to elaborate the optimal in vitro test for detection of chemicals interfering with GJIC, (inhibitors and enhancers) using human and animal cells, and to study mechanisms of GJIC and their role during the promotion phase of the carcinogenesis process.

T-1029 Evaluation of the Compounds Patulin, Penicillium Roqueforti Toxin, Botryodiplodin, and Palytoxin in the Metabolic Cooperation Assay. E. HONIKMAN-LEBAN and M.M. Shahin, Department of Chemical Protection and Photobiological Research in Vitro, L'Oreal Advanced Research Center, Aulnay-sous-Bois, 93600, France.

Mycotoxins are toxic substances produced by a number of fungal species. The aflatoxins are the most widely studied agents of this group. For example, aflatoxin B1 is a mutagen and one of the most potent hepatocarcinogens known to humans. Our present study was designed to investigate the three mycotoxins patulin (an antibiotic derived from the metabolites of a number of fungi), Penicillium roqueforti toxin (produced by Penicillium roqueforti), and botryodiplodin (produced by Botryodiplodin theobromae and by some strains of Penicillium roqueforti) as well as the compound palytoxin (produced by a marine organism, Palythoa) in the assay for gap junction-mediated intercellular communication. Inhibition of this form of intercellular communication by chemical agents is postulated to be a factor in tumor promotion. In this gap junction-mediated intercellular communication assay we use a co-culture of male Chinese hamster lung fibroblasts, wild type V79 cells (HGPRT+), sensitive to 6-thioguanosine monophosphate and mutant V79 cells (HGPRT-), unable to form the toxic metabolite 6-thioguanosine monophosphate. While the cells are grown in a 6-thioguanine selective medium, they are treated with nontoxic concentrations of the chemical being tested. The ability of the chemical to inhibit intercellular communication between both types of cells is then evaluated by calculating the percentage of recuperated colonies (6-thioguanine resistant cells). The results are compared with the negative (solvents) or positive (12-o-tetradecanoyl-phorbol-13-acetate, TPA) controls. None of the four compounds tested showed inhibitory effect in this metabolic cooperation assay. The data will be discussed at the forseen meeting.

T-1030 In Vitro Toxicological Evaluation of Zidovudine (ZDV), Itraconazole (IC) and Rifabutin (RIF) alone and in Combination. B.C. FOSTER, M.A. Bayne, D.L. Wilson, and S.R. Khan. Health Protection Branch, Health and Welfare Canada, Ottawa, Ont, Canada, K1A 0L2.

Drug therapy is dependant upon the beneficial pharmacological activity of drugs. However, many of the drugs used, or proposed for use in patients with AIDS are cytotoxic. Since these drugs are often given concomitantly with other therapeutic agents it is important to determine if there will be adverse effects on the lymphocytes which would further impair the immune response. Epstein-Barr virus transformed (GM 9948) and Con-A stimulated peripheral blood human leukocytes were incubated with IC (1 μg), RIF (1 μg), ZDV (0.5 μg), IC/ZDV, and RIF/ZDV added at t=0 h. ^{3}H-Thymidine (THY) was added alone or with 1, 5, 10 μg/mL cold THY at 0 or 72 h. Cells were harvested at times up to 96 h, counted and assayed for viability, ^{3}H-THY, BUN, dextrose, glutathione (GSH), and protein. In cultures where ^{3}H-THY was added at t=0 h, uptake after 24 h decreased with increasing levels of THY; but was directly proportional to the amount of ZDV in the 1 and 5 μg/mL THY cultures. However, ZDV reduced the uptake of ^{3}H-THY but did not effect cell count or viability in 96 h cultures. IC had a greater effect on reducing cell count, GSH, viability and uptake in GM 9948 than with IC or ZDV alone. RIF had a variable effect on cell count and viability of T cells, and may mediate the effect of ZDV on ^{3}H-THY uptake. IC and RIF altered the transepithelial resistance of human cells grown in Millicell™ inserts. These studies suggest that transformed cells can provide complementary information on the effects of these drugs, and studies with multiple end points are required for full toxicological evaluation.

T-1031 **TESTING CONTACT LENS SOLUTIONS ON SERIALLY CULTURED CORNEAL CELLS,** Peter A. Simmons, Ph.D., James O. LaMotte, O.D., Ph.D., Christopher Orenic, B.A., Southern California College of Optometry

Corneal epithelial cells isolated from rabbits were grown in tissue culture in a supplemented medium designed to stimulate cell proliferation. After subculturing 2-3 times, established corneal cells were photographed at arbitrary locations in each culture well, and then the cells were exposed for 1 hour to preserved and unpreserved saline products designed for the rinsing and storage of contact lenses. Additional photomicrographs were made 1 day, 3 days, and 7 days following exposure, allowing measurement of numbers of surviving cells and changes in cellular morphology. In general, cultures exposed to ReNu, Optifree, or Boston Advance solutions survived exposure and continued to proliferate. Cultures exposed to Wet-N-Soak Plus and Lens Plus showed fewer surviving cells. This method of testing toxic effects of contact lens solutions has distinct advantages over animal testing as it allows greater control of testing conditions, is extremely sensitive to detrimental effects, and does not require use of live animals. Supported by the In-Vision Institute.

T-1032 **The Trans-Epithelial Permeability Assay as an *In Vitro* Assay for Predicting Ocular Irritation of Surfactant Formulations.** **K. M. Martin and C. W. Stott. Johnson and Johnson Consumer Products Inc., Skillman, NJ. 08558**

The Trans-Epithelial Permeability (TEP) Assay has been evaluated as an indicator of ocular irritation for surfactant formulations. *In vivo*, the corneal epithelium serves as a relatively impermeable barrier protecting the underlying stroma from potential irritants. Breakdown of this barrier may lead to corneal opacity *in vivo*. The permeability barrier depends on tight junctions and desmosomes formed between adjacent cells. These junctions are also found in several established epithelial cell lines including Madin-Darby Canine Kidney (MDCK) cells. Confluent monolayers of MDCK cells grown on microporous filters were exposed to 28 surfactant formulas. The injurious effect of these formulas to the permeability barrier after 15 minutes was measured by the leakage of sodium fluorescein through the epithelial layer. EC_{50} values were generated by probit analysis. The log EC_{50} value was compared to the data obtained from the rabbit eye test. The correlation to the rank classification is 0.73 and the correlation to the percent corneal opacities is 0.77. Correlations involving *in vivo* results determined largely by corneal reactions correlated with the TEP better than reactions in other regions of the eye. The TEP assay seems to be a good model for predicting corneal damage. Using this test, moderate to severe corneal irritants could be eliminated from *in vivo* testing based on an EC_{50} value of 2% or less. Using this cutoff, thirteen formulations classified as slight or slight to moderate irritants using the *in vivo* data were correctly predicted as less than moderate (negative) by the TEP assay. Thirteen formulations classified as greater than moderate irritants in the *in vivo* data were correctly predicted as moderate or greater irritants (positive) using the TEP. Two formulas, one predicted as slight and the other a slight to moderate irritant by the *in vivo* data were incorrectly predicted as positive irritants. Based on these values the TEP has a sensitivity of 0.87, a specificity of 1.00, a positive predictive value of 1.00 and a negative predictive value of 0.87.

T-1033 Agar Diffusion Cytolysis: An Alternative Screen for the Prediction of a Corrosive Ocular Response. D. A. LASKA, J. T. Reboulet, J. O. Houchins, and R. L. St. Clair. Toxicology Research Laboratories, Lilly Research Laboratories, A Division of Eli Lilly and Company, Greenfield, IN 46140

Manufacturing intermediates and formulation components constitute the bulk of compounds evaluated in in vivo ocular irritation models. Our laboratory has evaluated an alternative method which employs a monolayered SIRC cell culture (rabbit cornea fibroblast, ATCC CCL60) as an initial screen in an ocular tier testing scheme. The assay is specifically designed to identify those compounds with corrosive potential, thus lowering the chance of testing corrosive materials in animals. Twenty four hours after plating 100mm dishes with a density of cells to yield a monolayer upon attachment, the culture medium is replaced with a 0.01% neutral red /1% agar overlay and incubated for an additional 24 hours. Both solid and liquid compounds (tested as supplied) are applied atop the agar overlay in glass cylinders or on paper discs, respectively, at doses of 2, 5, 10, and 25 mg/µl and incubated at 37°C for 24 hours. Clear zones resulting from the loss of the neutral red dye following compound-induced cytolysis is measured, and the dose response is compared to the response of positive and negative in vitro controls. Cytolysis data, from nine formulation components and 13 chemically complex manufacturing intermediates tested thus far, has correlated well with the in vivo data. The assay has demonstrated the following performance characteristics: Sensitivity (proportion of corrosives properly identified), 89%; Specificity (proportion of negatives properly identified), 92%; with an overall Predictive Value of >90%. Ongoing efforts in the laboratory include refinement of data interpretation for the prediction of moderate to severe ocular irritation potential.

T-1034 Comparison of the Responses of Human Skin to the Responses of the Living Skin Equivalent to Two Classes of Surfactant. R. GAY, M. Swiderek, A. Ernesti and A. M. Kligman[1]. Organogenesis Incorporated, Cambridge, MA 02142 and [1] Department of Dermatology, University of Pennsylvania, Philadelphia, PA 19104.

Living skin equivalents (LSE ™) are commercially available co-cultures of human dermal fibroblasts in a collagen lattice with overlying layers of stratified human epidermal keratinocytes. LSE were used to evaluate the relative dermal irritation potentials of selected anionic and cationic surfactants using a variety of endpoints. Time and dose-dependent changes in cellular viability, morphology, water permeation, and in the release of the proinflammatory mediators, prostaglandin E2 and interleukin-1-alpha, were determined. For the five anionic surfactants tested the time required to inhibit mitochondrial function by 50%, as assessed by the thiazolyl blue (MTT) cytotoxicity assay, compared favorably to the rank ordering established by 24 hour occluded patch tests in humans. The capacity of these surfactants to disrupt the partial barrier function of the LSE stratum corneum was assessed by measuring the rates of tritiated water penetration through the LSE These results were compared to rates of transepidermal water loss on human volunteers exposed to these same surfactants. The variety of endpoints generated using the LSE illustrates the unique toxicological character of these classes of surfactant and emphasizes the advantages of using multiple endpoints for evaluating dermal irritation potential in this in vitro skin model.

T-1035 Comparison of several methods employed in in vitro cytotoxicology/growth assays. T.K. JOHNSON, Cell Culture Department, Sigma Chemical Co., POB 14508, St. Louis, MO. 63178.

The increasing emphasis on finding alternatives to in vivo animal testing in toxicological applications and the need for rapid preclinical evaluation of new drugs is spurring interest in the use of in vitro cytotoxicology and cell growth assays. A number of cellular parameters including lysosomal activity, mitochondrial activity, membrane integrity, irritation, and biomass can be employed to monitor the response of cells to various compounds. As has been noted by several authors, not all of these parameters exhibit comparable responses to the same test material. The use of a battery of tests has been suggested to evaluate the effects of unknown materials in order to maximize the likelihood of detecting a response. There have been several reports correlating the in vitro cytotoxic response with results of established in vivo animal tests such as the Draize eye irritation test. While a number of test methods have been employed to measure these cellular parameters no comparison of these methods has been reported. Quantitative data derived by different assay methods is difficult to compare. This is due in part to different sensitivities of the assays employed as well as intrinsically different levels of enzyme activity, mitochondrial activity and biomass on a per cell basis associated with different cell lines and cell types. We have compared the data generated for comparable cell numbers of various cell lines by different assay methods including MTT, XTT, acid phosphatase and neutral red. The effect of cell number in the assay procedures was also examined.

T-1036 Arsenic Induced Chromosome Changes in Cultured CHO Cells. W. HOWARD, S. Hoffman, and T.S. Kochhar. Kentucky State University, Frankfort, KY 40601.

For a long time arsenic compounds have been noted to cause cancer of skin and lung in humans. Cytogenetic studies conducted on the influence of these compounds have been controversial. To check into this further, the effect of arsenite and arsenate on chromosome aberrations and sister chromatid exchange (SCE) in cultured Chinese hamster ovary (CHO) was investigated. Exponentially growing CHO cells were treated with 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M of $NaAsO_2$ (tri-valent) and Na_2HAsO_4 (pentavalent) for 24hr at 37°C. It was noted that both arsenite and arsenate compounds caused an increase in the frequencies of chromosomal aberrations. The increase was essentially dose dependent. The most profound effect was noticed in 10^{-5}M $NaAsO_2$ where an excessive number of cells showed polytene chromosomes. The arsenicals used in this investigation also raised the rates of SCE but the increase did not appear to be dose dependent. All concentrations of chemicals tested showed approximately two-fold increase in SCE compared to the controls. The results definitely indicate a link between arsenicals and mammalian genetic material, however, additional studies are needed to show whether trivalent arsenic is the "ultimate carcinogenic metabolite" -- as indicated by previous workers.

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T-1037 In Vitro Effects of Heavy Metals on the Nonspecific Defense Mechanisms and Immune Response of Fish. G. JENEY, D. Anderson* and Z. Jeney. Fisheries Res. Institute, Szarvas, Hungary; *National Fish Health Res. Lab. Kearneysville, WV 25340 USA

Culture of rainbow trout (Oncorhynchus mykiss) spleen sections showed effects of heavy metals selenium, cadmium, arsenic, and copper on nonspecific defense mechanisms and specific immune response. Sections, 5 mm in diameter, were incubated in metal concentrations ranging from 0.1 ug/ml to 100 ug/ml with a Yersinia ruckeri O-antigen as the immunogen. After 10 days incubation, cells were separated and suspended for analysis. The phagocytic index and neutrophil activity assays showed no suppressive effects of the presence of the metals occurred at the lower concentrations; however, at 10 ug/ml, activity was greatly reduced. Neutrophil assays showed that cadmium gave a slight positive effect (rather than inhibition) at 0.1 ug; at 1.0 ug inhibition was seen in all samples containing metals. Leukocyte numbers were depressed in all samples above 1.0 ug/ml. Specific immune response assays, showing numbers of plaque-forming cells, reflected patterns of the nonspecific defense assays. In vitro immunotoxicological assays are developing for environmental monitoring of fish, giving more rapid results while using fewer animals. Also, they allow greater control of temperatures, critical in immunological tests of poikilotherms.

T-1038 Development of a Cell Line of Hepatocytes from Atlantic Menhaden (*Brevoortia tyrannus*). M. FAISAL, S. Sami, B.J. Rutan, D.E. Holmes, and S.F. Hoegerman. Virginia Institute of Marine Science and Department of Biology, College of William and Mary, Gloucester Point, VA 23062.

The liver is the main target organ for toxic chemicals in all vertebrates including fish. Despite previous serious effort, no permanent cell lines of hepatocytes have been developed. Recently, we have standardized a single-step enzymatic digestion procedure to obtain long-term culture of liver cells from marine fish. A few proliferating cells from a culture of Atlantic menhaden liver formed colonies that later joined to form a confluent monolayer. Eighty eight subcultures were obtained within 30 months with a doubling time of 48 hrs. The cell line, designated as AML, has the typical morphology of hepatocytes as revealed by light and electron microscopy. Over 85% of the AML cells possess highly refractile droplets that stained positively with Sudan Black B, suggesting a lipid nature, granules that are positive to naphthol AS-D chloroacetate esterase staining, and form formazan upon stimulation with bacterial extracts. AML has 43 telocentric chromosomes, 2 sub-, and 2 metacentric chromosomes. AML cells produce glucose upon glucagon stimulation. Moreover AML cells contain measurable levels of enzymes known to be present in hepatocytes. Based on these findings, AML cells are apparently derived from hepatocytes.

T-1039 Macromolecular Synthesis in Cultured Hepatocytes of Spot (Leiostomus xanthurus) from Polycyclic Aromatic Hydrocarbon Contaminated Environment. N.J. MORSE, M. Faisal, and R.J. Huggett. Virginia Institute of Marine Science, The School of Marine Science, The College of William and Mary, Gloucester Point, VA 23062.

It has been shown that many pollutants including polycyclic aromatic hydrocarbons (PAH) can interfere with biochemical and physiological pathways in exposed fish. For example, fish residing in PAH-contaminated sites of the Elizabeth River (e.g Station-217, 500,000 μg PAH/kg dry sediment) display a relatively high incidence of diseases including eye lens cataract and of cancer. The extent of toxic chemical effects upon the cellular material of spot liver was therefore followed over the summer of 1991 by examining the rate of synthesis of the main cellular macromolecules; DNA, RNA, and protein. Primary hepatocyte cultures were initiated from liver of spot caught from Station-217 of the Elizabeth River (ER) and the relatively clean York River (YR, Pages Rock, PAH levels <1000 μg/kg dry sediment). DNA synthesis rate of ER fish hepatocytes showed a 5-fold increase as compared to that of the YR fish hepatocytes. RNA and protein synthesis were both suppressed by 36% when exposed to the PAH environment of the ER. Since DNA synthesis displayed the greatest degree of effect, thereafter, it was used as an indicator of effects in subsequent samples. It was found that ER fish hepatocytes exhibited a 15-fold increase in DNA synthesis compared to the YR fish hepatocytes. Similarly, in vitro exposure of spot hepatocytes to benzo(a)pyrene (a common PAH found in Elizabeth River sediment) induced a further increase of 27% in the ER spot hepatocytes while YR spot hepatocytes exhibited a 37% suppression. When Elizabeth River fish were separated according to presence or absence of cataracts, the rate of DNA synthesis in cataractous fish was 4 times greater than that in noncataractous ER fish. These results suggest that biochemical or physiological processes of fish are effected by PAH among other contaminants of the Elizabeth River.

T-1040 In vitro Culture, Differentiation, and Macromolecular Synthesis of Eye Lens Epithelium of Cataractous and Non-cataractous Spot (Leiostomus xanthurus). C.D. WILLIAMS, M. Faisal, and R.J. Huggett. Virginia Institute of Marine Science, The School of Marine Science, College of William and Mary, Gloucester Pt., VA 23062.

High prevalence of eye lens cataract in fish sampled from waters contaminated with toxic chemicals has been reported. For example, 50% of all sciaenids sampled from a site in the Elizabeth River, VA, whose sediments are highly contaminated with polycyclic aromatic hydrocarbons (PAH), exhibited different degrees of eye lens cataract. The underlying mechanism for cataract development has not been investigated due to the lack of an in vitro cellular system. Following extensive research, the authors were able to culture, maintain, and subculture (up to 12 passages) fish eye lens cells from the marine teleost, spot. Primary cultures consisted of clusters of adherent elongated cells that joined to form a confluent monolayer. Cells were heterogeneous in shape, size, and appearance and were relatively slow to grow to confluency (doubling time of 200 hr. at 27°C and 5% CO_2). Several cell types were morphologically similar to those described for epithelial cells and fiber cells of intact lenses. Furthermore, some cells demonstrated phagocytic activity. Cytospin smears showed cellular morphologies that ranged from small (5 μm) with a high nucleus:cytoplasm ratio to large cells (100 μm) with a low nucleus:cytoplasm ratio. The largest cells contained no nuclei, consisted mainly of a circular, homogenous, and translucent, structureless "lentoid body(ies)" that occupied most of the cell. This apparent progression of eye cell appearances seems to indicate a differentiation of epithelial cells (replicating cells) to fiber cells formation (crystalline producing cells). Eye cells derived from cataractous as well as non-cataractous Elizabeth River spot exhibited similar culture characteristics but subsequent subculture proved unsuccessful. Furthermore, comparison of in vitro radiolabeled precursors employing York and Elizabeth River spot eye cells has been performed. Cells from cataractous eyes demonstrated an increase of six-fold and nine-fold of DNA and RNA synthesis respectively as compared to the non-cataract eye cells. Protein synthesis, measured by ^{3}H-leucine uptake, showed no differences between any of the eye lens cells. Results show that PAH exposure may interfere with some cellular processes of the fish eye lens.

T-1041 CELLULAR TOXICOLOGY

Chromium toxicity in N. crassa

Many heavy metal ions are toxic to cell growth at higher concentrations due to a) their interference with the uptake and utilization of essential metal ions, or b) to their ability to complex with cellular macromolecules.

Chromium ions have been reported to be toxic as well as carcinogenic under certain conditions. Exposure to chromium, industrially, can be in either hexavalent or trivalent form. But hexavalent chromium, like several other metal ions, binds to DNA only after its reduction to the trivalent state.

Since little is known regarding toxicity of different forms of chromium to any single organism, it was considered of interest to examine this aspect in N. crassa, wherein mechanisms of toxicities of a variety of metal ions have been extensively studied. In this mold, toxicities of heavy metal ions are annulled by Mg^{2+} or Fe^{3+} but by two completely different mechanisms. Mg suppresses toxic metal ion uptake. Metal ions interfere with synthesis of iron dependent enzymes within the cell, and this inhibition can be counteracted by excess supplemented iron; control of toxic metal ion uptake is not involved in this case.

The present studies have shown that Cr^{6+} (as dichromate) primarily interferes with sulphate uptake and utilization for sulfur amino acid biosynthesis; excess sulfate can suppress Cr^{6+} toxicity. On the other hand, Cr^{3+} is toxic by being, in N. crassa, the most powerful inhibitor of Fe^{3+} metabolism, among all toxic metal ions examined todate. Its toxic effects manifest as enhanced excretion of an iron binding siderochrome and reduced growth, are completely counteracted by supplementation with fairly low levels of Fe^{3+}.

This study constitutes the first instance wherein, in the same organism, two different valence states of chromium have been shown to be toxic by two completely different mechanisms. They further demonstrate that, in N. crassa cells, Cr^{6+} is not reduced from its hexavalent state.

T-1042 A number of pyrazolines and their derivatives are known to possess antibacterial, antifungal and antiviral activity. Some fluorinated heteroaryl pyrazolines have also been reported as potential biocidal agents. the N-heterocyclic nucleus adjacent to a carbonyl group appears to be responsible for significant biological activity in pyrazolines. Such a functionality is invariably present in all the new Bis (2-pyrazolin-3 yl) benzenes and pyridines reported from our laboratory recently. In the present work the antimicrobial activity against bacteria and fungi of these compounds has been investigated. The anibacterial activity was determined against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, pseudomonas auerginosa, Proteus vulgaris and Candida albicans. The antifungal activity was determined against fungi Curvularia lunata, Fusarium solani and Helminthosporium oryzae. All these compounds exhibit considerable antifungal activity, It is interesting to note that significant activity is exhibited by the compounds with a halogen as substituent in the phenyl moiety.

T-1043 A Quantitative in vitro Test for the Detection of Waterborne Toxins. J.J.ALEXANDER and E.M.Bey, Department of Microbiology, University of the Witwatersrand, P.O. WITS, 2050, Johannesburg, South Africa.

The most reliable general test for toxic pollution of water has been the fish test. However, this test is time consuming, and not readily available. We have developed a rapid in vitro test which is designed to replace the fish test as a convenient and general method. Metabolic activity after exposure to test water samples, is reflected as a proportion of the activity measured in control cells exposed to known clean water samples. McCoy cells, seeded in 96 well tissue culture plates, are exposed to test water samples serially diluted in growth medium and incubated for 24 hours. Thereafter 20uℓ of a 5mg/mℓ solution of a tetrazolium salt is added and incubation continued for 4 hours. The cells are fixed, the supernatants removed and isopropanol is added to solubilize the formazan granules in the cells. The absorption value in each well is read on an ELISA plate reader, using the appropriate filter, and compared with control sample readings. This test can detect the presence of 0.1ppm of nickel added to known clean water where 75% of control absorbance readings are obtained; at 1ppm 50% readings and at 2ppm 25% readings were found. This test measures the additive or synergistic effect of potential toxins; it may be a proactive predictor of future toxic levels using concentrated samples; without knowing the specific toxin/s, the probable source of pollution may be identified. The test has the potential to detect xenobiotics detrimental to the metabolism of living organisms.

V-1001 Urothelial Cell Growth In Vivo On Biodegradable Polymer Scaffolds, A. ATALA, J. P. Vacanti, A. B. Retik, and M. R. Freeman. Urology Laboratory, Children's Hospital and Harvard Medical School, Boston, MA 02115.

We have developed a novel system for the delivery and growth of rabbit urothelial cells in athymic nude mice on polyglycolic acid polymers (PGAP). The purpose of this study was to determine the proliferative potential and differentation state of rabbit urothelium in PGAP scaffolds implanted in host animals for 5 to 50 days. Urothelial cells were collected by collagenase treatment of ligated rabbit bladders or by explant of mucosal surfaces directly onto PGAP scaffolds. After a brief culture period the cell-polymer scaffolds were implanted into the mesentery, omentum or retroperitoneum of athymic mice. Animals were sacrificed at 5, 10, 20, 30 and 50 days after implantation and implants were retrieved and evaluated using histologic, immunocytochemical and immunoblot methods. Vascular ingrowth was evident in most implants by 5 days. Hematoxylin and eosin staining and anticytokeratin immunohistochemistry demonstrated the presence of epithelial cells in some implants at all time points. Urothelial cell proliferation was suggested by the formation of epithelial sheets at later time points and was confirmed by in vivo BrdU labeling and anti-BrdU immunodetection. Maintenance of urothelial differentation was indicated by immunoblot detection of urothelial-associated cytokeratins and immunohistochemical detection of uroplakin, a urothelial cell surface protein. Progressive degradation of the PGAP occurred coincident with expansion of the urothelial cell population. These findings suggest that cell populations of normal urothelium can be expanded by cultivation on PGAP scaffolds in vivo.

V-1002 Expressed-differentiated properties of rabbit kidney proximal tubule cells in primary culture grown in hormonally-defined medium in total absence of glucose and insulin.
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Rabbit proximal tubule cells (RPTC) in primary culture retain many features of this nephron segment. However, they revert from gluconeogenesis to glycolysis. To determine whether glucose and insulin deprivation, two components usually present at high concentration in the culture medium, could prevent this metabolic conversion without the loss of differentiated properties, RPTC were cultured in hormonally-defined medium, free of glucose and insulin ([-I-G]medium) and compared to RPTC cultured in medium supplemented with 17.5 mM glucose and 5µg/ml insulin ([+I+G] medium). In both culture conditions, RPTC grew at a similar rate and confluency was reached at day 7 . Expression of differentiated properties assessed from day 0 to day 11 such as Na^+ dependent glucose transport, apical and basolateral membrane enzyme activities, glutathione-S-transferase, N-acetyl-β-D-glucosaminidase and succinate dehydrogenase activities were not significantly different in RPT cells cultured in [+I+G] and [-I-G] media. In both culture conditions, ATP content remained high and stable from day 2 to day 11. Phosphoenolpyruvate carboxykinase and fructose1,6-biphosphatase activities decreased dramatically throughout the culture period whatever the culture medium used. Hexokinase, pyruvate kinase and lactate dehydrogenase activities increased markedly from day 0 to day 11 in RPTC grown in [+I+G] medium whereas in RPTC cultured in [-I-G] medium they increased to a smaller extent until day 7 and remained unchanged thereafter. RPTC grown in [-I-G] medium produced lactate at a constant rate of 0.15 µmol/hour/mg protein from day 4 to day 11 while this rate was 3 fold higher at days 4 and 7, and 6 fold higher at day 11 in RPTC grown in [+I+G] medium.
In conclusion, glucose and insulin are not essential factors for RPTC growth and differentiation. Total glucose and insulin deprivation from the culture medium limits the orientation towards glycolysis but does not prevent the decrease of gluconeogenic enzyme activities.

V-1003 Extracellular Matrix Analogs as Growth Factor Delivery System. C.J. DOILLON and F. Roy, Laval University and Saint-François d'Assise's Hospital, Research Center (D00-744), 10 rue de l'Espinay, Québec (Québec), Canada, G1L 3L5.

During wound healing, numerous interactions occur between extracellular matrices (ECMs) and growth factor (GFs) at specific cell membrane receptors. It has also been shown that cells require continual exposure to GF to divide. Collagen sponge-shape matrices can be designed as wound dressings and be used to deliver GF into wounds. In order to optimize wound dressings, we have investigated in vitro the effects of various ECM analogs and GFs. Using a serum-free culture medium model, human fibroblasts were cultivated on different supports made of collagen, hyaluronic acid, chondroitin sulfate, fibronectin and/or fibrin in which specific GFs were incorporated. The release of GFs from ECMs was characterized by immunochemistry and radiolabeling techniques. A slow delivery was observed from fibrin, fibronectin and collagen. As measured by cell counting and ^{3}H-thymidine incorporation, cell replication was significantly increased for 24 hours on fibrin containing fibroblast GF (FGF) or epidermal GF (EGF) when compared to control groups. FGF activity was sustained for 48 hours when FGF was mixed with fibrin. The incorporation of platelet-derived GF (PDGF) within a mixture of fibrin and hyaluronic acid or fibronectin slightly improved cell replication at short term while within hyaluronic acid or fibrin cell replication was stimulated at long term. Fibrin seems to play an important role to carry and deliver GFs particularly FGF. This feature can be used to modify cell behavior on synthetic and/or biological biomaterial surfaces and porosities.

V-1004 NASA Bioreactor: A Unique In Vitro Fluid Culture System for Three-dimensional Tissue Formation with Anchorage-dependent Cells. [1] S. R. GONDA, [2] G. M. Marley, [3] N. A. Schroedl, [4] R. S. Tuan, [4] R. K. Sinha, [5] M. Ingram, [1] NASA Life Sciences Division, Washington, D.C, 20546, [2] NASA Johnson Space Center, Houston, TX 77058, [3] Alfred I duPont Institute, Wilmington, DE 19899, [4] Dept. Orthopaedic Surgery, Thomas Jefferson University, Phila., PA, 19107, [5] Huntington Medical Research Institutes, Pasadena, CA, 91101

The NASA bioreactor, designed to simulate some aspects of microgravity on the ground and to be compatible with operation in space aboard the Space Shuttle and Space Station Freedom, provides a hydrodynamically low-shear culture environment that has been successfully utilized for the three-dimensional growth of mammalian cells. This culture system overcomes gravity-induced limitations of shear associated with fluid spinner cultures or limitations of gas and nutrient diffusion in agar cultures. The bioreactor has been successfully used to culture anchorage-dependent human and non-human primary cells, cell lines, and neoplastic cells with or without microcarriers. Studies with three different cell types will be presented describing (i) the in vitro growth and differentiation of primary adult rat muscle satellite cells into three-dimensional contracting myotubes, (ii) the in vitro growth and development from single human glioma cells of large (>2.5 mm^3) tumor spheroids that morphologically and histologically resemble biopsied in vivo patient gliomas, and (iii) the in vitro formation and mineralization of three-dimensional osteospheroids from single cells of human or rat osteosarcoma.The ability to reproducibly culture in an in vitro fluid environment, large three-dimensional cellular aggregates that structurally and functionally more closely resemble their in vivo state, indicates that this bioreactor system offers significant advantages and great utility for conducting basic and medical research studies.

V-1005 In Vitro Analyses of Wound Healing Promotion by a Human Dermal Replacement. S.R. Slivka, L. LANDEEN, F.S. Zeigler, and , R.L. Bartel, Advance Tissue Sciences (formerly Marrow-Tech), Inc., La Jolla, CA 92037

A human dermal replacement (Dermagraft^{TM}) was developed for the treatment of burns and skin ulcers. To produce this dermal replacement, fibroblasts derived from neonatal foreskins were isolated and expanded in monolayer culture. Fibroblasts were then grown on a biodegradable mesh for 18 days and cryopreserved at -70 C. Fibroblasts on this mesh formed a three-dimensional structure consisting of fibroblasts surrounded by an extracellular matrix rich in fibronectin. In an ongoing clinical trial, dermagraft, used with meshed autografts, was found to "take" in the wound bed, vascularize rapidly, and support epithelialization. Here we describe in vitro methods to analyze the mechanisms of wound healing promotion by this dermal replacement. Lysate from the dermal replacement was mitogenic for serum starved dermal fibroblasts. This lysate contains bFGF, as demonstrated by immunoblotting, which may be in part responsible the mitogenic activity observed. Characterization of other growth factors in this lysate is currently underway. To evaluate epithelialization, foreskin explants were placed on the dermal replacement. These explants "take" and the epidermal outgrowth from these explants was evaluated by conventional histology. We are studying modulation of the extracellular matrix of the dermal replacement by altered growth conditions. Explant cultures will be used to evaluate the effect of altered extracellular matrix on epidermal outgrowth.

V-1006 Optimization of Antigen production by HTLV-I cell lines grown under serum and serum free supplemented conditions. A.V. Trevino, M. Pollman, C. Brooks, L.E. Mathes, and M.D. Lairmore. Department of Veterinary Pathobiology, The Ohio State University, Columbus, OH 43210

Human T-lymphotrophic virus type I (HTLV-I), etiologic agent of adult T-cell leukemia (ATL), has become a target for retroviral vaccine development. Previous observations suggested serum starvation places stress on retroviral infected T-cells resulting in producton of incomplete viral proteins (Olsen et al., Leukemia: Recent Advan. Bio. & Treatment, pp 239-248, 1985), and increasing the production of virions - precursors which may enhance the immunogenicity of potential vaccines (Geelen et al., J. Gen. Virol., 69:2913-2917, 1988). Initial comparative analysis of HTLV-I cell lines for optimal production of antigens under normal and stressed conditons, as well as, growth characteristics were examined for this purpose utilizing MT-2 as the standard cell line. HTLV-I infected cell lines (MT-2, HuT-102, and C10-MJ) cultured in RPMI with 15% FCS were inoculated into 480cm^2 roller btls. at 1 x 10^8 cells to a volume of 250 ml RPMI with 15% FCS in triplicate, gased in 37°C CO_2 (5%), and after 2 hrs. transfered to 37°C warm room for 3 days incubation (roller at 0.5 rev./min). Three roller bottles were harvested each day, supernatants collected for HTLV-I p24 Ag assay, and viable cells counted. As a reference for serum-free analysis, the average MT-2 total cell concentration of day 3 was used (1.74 x 10^8 cells in 250 ml serum-free media) and the procedure repeated with harvests on days 1, 3, & 5. All samples decreased in viability during normal serum growth conditions, yet total cell concentration increased in MT-2 (2 fold) and HuT-102 (3 fold) while C10-MJ had only slight growth after 3 days. Serum-free conditions produced a slight decrease of viable cells on day 1, then approximately 35% and 45% decrease on days 3 and 5, respectively, in all cell lines. Also, total cell concentrations increased on day 1, yet went unchanged there after. Antigen production increased slightly during normal serum growth conditions (MT-2, 6 ng/250ml; HuT-102, unchanged; C10-MJ, 15 ng/250ml on day 2, and HuT-102, 10 ng/250ml; MT-2 and C10-MJ unchanged on day 3), yet C10-MJ produced the greatest quantity of antigen when calculated on a per cell basis. C10-MJ, under serum free conditions, also produced the greatest amount of viral Ag per cell. Under serum-free conditions, the major concentration of viral Ag was produced between days 1 and 3 in all three cell lines and viral proteins were frequently detected by western blot assay. Thus, 1) C10-MJ proved to be the optimal viral Ag producer under both serum and serum-free conditions and an ideal cell line for vaccine preparations, and 2) serum deprivation may alter HTLV-1 protein-process that can result in increased precursor proteins. Commercialized serum-free supplemented medium (AIM-V, Gibco) supports growth of MT_2 cells with a doubling time of 2 days with excellent viability (90%). These growth conditions may assist in production of viral Ag without interference from normal serum constituents for future vaccine studies.

V-1007 A Novel In Vitro Model for Human Prostatic Cancer. J. T. Mendoza and A. L. SPIERING. St. Joseph Hospital Cancer Research Laboratory, Houston, TX 77003 (JM) and Synthecon, Inc., Friendswood, TX 77546 (ALS)

The rotating-wall vessel (RWV), a new tissue culture technology developed by NASA's Johnson Space Center, has been utilized in preliminary studies on prostatic cancer. The RWV consists of a horizontally rotating cylinder with an oxygenator core attached to a rotating base within the confines of a standard CO_2 incubator. The low shear effects of this vessel enable us to produce high density cultures (8-11 x 10^6 cells/ml) as well as to produce co-cultures which develop into three dimensional tissue-like structures. We have successfully cultured human prostatic mesenchymal and epithelial cells in the RWV. Co-cultures of these two cell types by conventional tissue culture methods results in cellular responses more closely resembling those observed in vivo. Co-culture in the RWV allows for growth in three-dimensions, bringing the in vitro environment one step closer to the in vivo condition. The ability to develop such cultures in the RWV provides a model system for investigating the cellular basis of prostatic cancer. Supported by The Stehlin Foundation for Cancer Research and Synthecon, Inc.

V-1008 Human mesothelioma cell line ZK 70, showing both, endothelial and epithelial differensation within protein free medium. K-H. BERGHÄUSER B. Knoblauch, S. Menke, H. Burger, A. Schulz. Institute of Pathology, Justus-Liebig-University, Giessen, Germany.

Presently human mesothelioma cells are cultured in vitro by different scientific groups. Usually cells are grown in media containing growth factors or the black box fetal calf serum likely to contain a mixture of different growth factors. This additive is the most relevant influence disturbing studies on cell-cell interactions as influenced by secreted factors. A pleural effusion punctured from a patient with pleural mesothelioma was taken in culture and characterised, showing endothelial and epithelial immunocytochemical markers and various cytogenetic changes in chromosomes 1,11,12,13,15 and 19. These findings were constant over 280 passages. Further nude mice passage and soft-agar passage showed constant tumorgenity. After the 220th passage tumor cells were changed on RPMI with added L-Glutamine only and thus serum free medium. Tumor cells double into 19 hours in this medium. This proliferating activity indicates the exsistence of autocrine growth regulation in this tumor, but Western blot analysis of media contents till now didn't reveal secretion of growth factors as PDGF, alpha and beta-FGF, EGF, TGFbeta and IL 2.

V-1009 Both the Substrate and the Cytoskeleton are Important in Directing Protein Secretion in MDCK Cells.
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We have investigated how the extracellular substrate can influence protein secretion, as well as basement membrane (BM) secretion by the epithelial cell line Madin-Darby canine kidney (MDCK). When MDCK cells are grown on plastic substrates they appear fibroblast-like and produce no BM. When cells are grown on microporous membranes, they appear more morphologically polarized and produce a distinct BM. Since MDCK cells grown under these two conditions have different cytoskeletal arrangements we asked if microtubules (MTs) or microfilaments (MFs) might contribute to the appearance of the BM. Inhibition of MTs by nocodazole treatment did not affect which proteins were secreted, but did affect the rate of protein secretion regardless of the substrate, and resulted in early BM secretion in cells grown on microporous membranes. Inhibition of MFs by cytochalasin D treatment diminished a 40 kDa and a 50 kDa protein in cells grown on both plastic and microporous substrates. However, there was an additional 135 kDa protein produced only in cytochalasin D-treated cells grown on plastic substrates. To investigate the cytoskeletal effects on the secretion of the BM protein laminin, immunoprecipitation of laminin was accomplished under the same culture and inhibitor conditions. Both nocodazole and cytochalasin D treatment resulted in the increased synthesis and/or retention of cellular beta laminin, while cytochalasin D alone resulted in the secretion of two different isoforms of alpha laminin. It can be concluded that alteration of the cytoskeleton of epithelial cells not only can affect the species of protein that is secreted but can affect the rate of protein release. Thus the cytoskeleton might be important in directing how or when a cell secretes a basement membrane.

V-1010 Effects of NGF and Gamma-IFN on the Diverse Subpopulations in the Neuroblastoma Cell Line: SY5Y. J. RIDGE, D. Terle and I. Levenbook.
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To determine the effects of nerve growth factor (NGF) and gamma-interferon (IFN) on neuroblastoma (NB) cells we selected SY5Y, a cell line with a predominantly neuroblastic phenotype. These cells have receptors for, and are responsive to, NGF; contain single genomic copies of the N-myc proto-oncogene; have the capacity for neurite extension in culture; and are responsive to differentiating agents. Using NGF, IFN and NGF+IFN treatment we found earlier that the combined treatment resulted in differentiation which was confirmed morphologically and immunohistochemically. Since NB is a neural crest-derived tumor, the cell line contains, along with neuroblasts, melanocyte and Schwann cell precursors. In the present study we looked at the effects of treatment on all three cell populations. With fluorescence activated flow cytometry, cells were analyzed for expression of the 200 kD neurofilament neuronal marker, the S-100 protein, a melanocyte and Schwann cell marker and myelin basic protein (MBP) produced only by Schwann cells. Cultures were grown for up to 15 days and examined at two sequential time periods: after 8 and 14 days of treatment. There was little or no difference in expression of the marker proteins between control (C) and NGF-treated cultures, whereas IFN and, to a greater extent, NGF+IFN-treated cells showed a significant increase, although the dynamics of marker expression differed. C and NGF cultures increased four to 20 fold over seeding densities from week one to week two while IFN and NGF+IFN cultures remained unchanged. Thus, our findings indicate that IFN and, even more so, NGF+IFN treatment induces differentiation in all the NB subpopulations concomitant with extensive inhibition of their proliferation.

V-1011 Regulation of Mesangial Cell Growth by Matrix and Soluble Proteoglycans. D.M. TEMPLETON, M.-Y. Fan and T. Miralem, Department of Clinical Biochemistry, University of Toronto, Toronto, Canada M5G 1L5

Mesangial cells (MC) of the renal glomerulus synthesize an extensive mesangial matrix that forms a portion of the glomerular capillary wall. Most MC in the healthy kidney are quiescent. Proliferation and over-accumulation of matrix are features of most progressive renal disease. We are using rat MC in culture to study the effects of matrix on the mitogenic responsiveness of these cells, and the potential role of mesangial proteoglycans (PG) as autocrine factors regulating these processes. MC in serum-depleted medium revert to G_0 phase of the cell cycle; immunohistochemistry shows that less than 5% express nuclear cyclin. On stimulation with serum, 95% express cyclin within 8 h and progress in synchrony to S phase with a burst of DNA synthesis at 18 h. G_0 cells constitutively incorporate [^{35}S]-sulfate into cell- and matrix-associated PG. Sulfation of these PGs follows DNA synthesis, doubling during S phase and subsequently declining to the quiescent level. In contrast, G_0 cells secrete very little newly sulfated PG into the medium, but this population increases continuously following stimulation until it accounts for a significant portion of total PG sulfation. Commercial heparin blocks the mitogenic response (half-maximal effect at 1 µg/mL), as does the unfractionated PG recovered from the medium of cycling cells. Heparin also effectively antagonizes other MC mitogens, including the growth factors PDGF, bFGF and EGF, when added at the time of stimulation, but not when the cells are blocked at the G_1/S interface with aphidicolin. Cells grown on plastic or Matrigel behave in a very similar manner in these assays. However, cells grown on type I collagen show increased spreading and a lower cell density at confluence. Incorporation of heparin into the collagen gel increases the confluent cell number. Whereas MC on collagen show a blunted response to mitogenic stimuli, they are more sensitive to suppression of the response by heparin. Therefore, the accumulation of interstitial collagen in the mesangium during pathological mesangial expansion may be expected to induce such phenotypic changes in the MC. We conclude that the MC is a useful model for studying the modulation of cell-matrix interactions by PGs.

V-1012 The Effects of Thermal Injury on Gene Expression and Ultrastructure of Rat Proximal Tubule Epithelium (PTE) In Vitro. N. Yamamoto, S.H. CHANG, I.K. Berezesky and B.F. Trump. Univ. of Maryland Sch. of Med., Dept. of Pathology and MIEMSS, Baltimore, MD 21201.

Thermal stress constitutes an important physical injury in mammalian systems involved in hyperthermia, burns, and therapeutic interventions. In addition to other effects, thermal injury induces a variety of "heat shock" genes and corresponding proteins, the function of which is, at least in part, protection of the target cells against additional injury and possibly uncontrolled division. We correlated the heat shock gene expression (hsp70) with changes in cellular ultrastructure in primary cultures of rat PTE. Cells were prepared from 4-6-mo-old male F344 rats by collagenase digestion followed by culture in DMEM/F12 with 10% FBS for 4 days and serum-starved for 2 additional days prior to experiments. Thermal treatment was at 42.5°C for up to 3 h with no recovery. At serial time intervals, RNA was isolated by RNAzol™ and cells fixed for electron microscopy (EM) in buffered glutaraldehyde/OsO_4. hsp70 mRNA increased within 15 min and peaked 70-fold at 2 h. EM revealed that control PTE were polarized with apical microvilli and junctional complexes, had well-developed Golgi and RER and many supranuclear lysosomes. By 1 h, there was condensation of mitochondria, simplification of microvilli, and early nucleolar segregation. By 3 h, mitochondrial condensation was marked, extreme prominence of Golgi apparatuses was observed, nucleolar segregation was prominent, and aggregation of intermediate filaments was present. By 9 h, the latter two changes became quite prominent. These studies clearly show that the induction of the heat shock response in rat PTE is accompanied by early cytoplasmic, nuclear, and nucleolar ultrastructural changes. (Supported by Navy N00014-88-K-0427.)

V-1013 The Substrate Can Affect the Net Synthesis and/or Retention of Laminin in NHEK Cells.
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Basement membranes are specialized extracellular matrices that separate various tissue compartments such as the epidermis from the dermis. The mechanisms by which basement membranes form are not known. We explored the possibilites that the type of underlying substrate coupled with the arrangement of the cytoskeleton can affect the secretion and/or retention of laminin. When Normal Human Epidermal Keratinocyte (NHEK) cells are grown on cross-linked type I collagen gels and type I collagen coated Millipore Millicell CM microporous cell culture inserts, they exhibit a marked increase in laminin B chain synthesis but not A chain synthesis as compared to conventional plastic grown NHEK cells. This increase in the synthesis of the B chain of laminin may be related to the substratum-directed arrangement of the cytoskeleton because the microtubule inhibiting drug, nocodazole, further increases the amount of laminin B chain detected. This observation is not apparent when NHEK cells are grown on conventional plastic substrata. We conclude that the substrate can influence laminin synthesis and/or retention and this may be, in part, regulated by the arrangement of the cytoskeleton.

V-1014 Histologic Patterns of Neoplastic Epithelia in Matrix and Gradient Culture.
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Finding of abnormal architecture in a biopsy is key for the pathologic evaluation of type and clinical severity of a tumor. Analysis in culture of the relationship between the initiating molecular insult and its expression as abnormal tissue has been slow, in part due to the absence of procedures for reconstituting the spatial imperatives observed in tissues in nature, i.e. growth in three dimensions of dense populations of cells with defined and recognized directions of metabolic exchange. This has been accomplished so that kinetic processes of normal and neoplastic epithelium can be reconstituted and analyzed. The appearance of NBT-II, rat bladder epidermoid carcinoma, in a collagen coated cellulose sponge matrix, is compared in a growth medium with and without a supplement of vitamin A. Illustrated in radial histophysiologic gradient culture chambers are human ovarian cancer, human bladder cancers, kidney cell line MDCK, and blood borne colonies of cancer cells in explants of the heart and the skin of chick embryos, i.e.the formation of metastases in culture. Cylindrical radial gradient culture chambers are easily made using a combined membrane of nylon mesh and collagen (In Vitro Cell. Dev. Biol. 27A:786,1991).

V-1015 The Human C/EBPα Promoter is Transactivated by a Related Protein, C/EBPβ.
D. R. Wilson[1], L. R. Hendricks-Taylor[1], S. C. Juan[2], and G. J. DARLINGTON[1,2]. Departments of Pathology[1] and Molecular Genetics[2], Baylor College of Medicine. One Baylor Plaza, Houston, Texas 77030.

The CCAAT/Enhancer Binding Protein (C/EBP) genes encode a family of closely related transcription factors that are expressed at high levels in the liver. These DNA binding proteins play a role in liver specific gene expression during development and differentiation. Furthermore, they are involved in the regulation of the hepatic response to inflammation. One member, C/EBPα, inhibits cell growth when overexpressed in adipocytes and hepatoma cells (Umek et al, Science 251:288-292, 1991). We have cloned the promoter of the human C/EBPα and have characterized regions that contain enhancer activity. Deletion analysis of the promoter linked to a luciferase reporter shows that positive cis-elements lie within 416 bp of the transcription start site. Coupling the fragment from -76 to -416 to a heterologous promoter (thymidine kinase) and cotransfecting with an expression construct containing C/EBPβ shows that this region of the C/EBPα promoter is transactivated by C/EBPβ. An expression vector for its own product, C/EBPα does not autoregulate in the same assay system. The definition of specific sequences in this site will be determined by footprinting and gel shift analysis.

V-1016 Optimization of TSH-Induced Gene Expression in Ovine Thyroid Gland Cells in Culture.
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Primary cultures of ovine thyroid gland cells respond to the addition of thyroid-stimulating hormone (TSH) to the nutrient medium by organizing into microfollicular structures and increasing the various iodine-metabolic functions characteristic of thyroid tissue. The magnitude of the TSH response is optimum when the cultures are initiated at a density of 10^6 cells/cm^2, the medium depth is maintained at 0.2 cm, the serum concentration is 20%, and the cells are prestimulated for 2 days with 1 mU TSH/ml medium, followed by 2 to 4 days in the absence of TSH and restimulated with 5-10 mU TSH/ml medium. These optimum conditions, based upon iodine-metabolic responses, were then used to examine other responses to TSH addition such as activation of adenylate cyclase, production of thyroid- specific gene products and specific messenger RNA levels. Under these optimum conditions TSH increased cyclic AMP 36-fold, synthesis of iodinated thyroglobulin 30- to 70-fold, and thyroid peroxidase activity 38- to 55-fold, relative to cells maintained in the absence of TSH following the prestimulation interval. Insulin (1μg/ml) synergistically increased the TSH responses. The resident population of messenger RNA molecules in TSH-stimulated cultures contained a 4- to 5-fold increase in thyroglobulin mRNA and thyroid peroxidase mRNA relative to control cultures. TSH addition resulted in a small increase in mRNA for the proto-oncogene *c-fos*, but not *c-myc*, at a density of 10^6 cells/cm^2. A small, transient increase in *c-myc* mRNA occurred 15 min after TSH addition to cultures initiated at a lower density of 10^4 cells/cm^2.

V-1017 Extracellular Matrix (ECM) Gene Expression by Fibroblasts In Vivo and In Vitro. S.A. BRUCE, A.M. Choi, M.H. Swee, and S.F. Deamond. School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, MD 21205

To analyze the role of cellular differentiation in the limited proliferative capacity of fibroblasts in vitro, we have determined the temporal pattern of expression of ECM genes by fibroblasts as a function of development and age in vivo and compared this with the expression pattern of the same genes by the same cells as a function of passage level in vitro. Previous ultrastructure studies [Exptl. Gerontol., 26:3-16, 1991] of Syrian hamster (SH) skin indicated that maximal secretory protein synthesis (e.g. collagen) began just prior to birth (15 days gestation (dg)) and continued until at least 1 month of age; in contrast, fibroblasts in 6 and 24 month adult dermis in vivo appeared quiescent in terms of collagen synthesis. To analyze more specifically which ECM proteins are produced at various developmental stages and ages, we quantitated the steady state levels of mRNA for fibronectin (FN), and the alpha-1 chains of procollagens types I and III (α_1I and α_1III). Total RNA was isolated from skin tissue, analyzed by Northern hybridization with radiolabelled cDNAs, and mRNA levels were quantitated by laser densitometric scanning of the autoradiographs. Maximal levels of the single FN transcript and the multiple α_1I and α_1III transcripts were seen in vivo between late fetal development (15 dg) and 2 mos of age. By 6 mos of age there was a general down-regulation of all of these genes in vivo. By comparison, the steady state levels of these mRNAs exhibited multiphasic patterns as SH fetal dermal fibroblasts progressed from low passage to high passage (senescence) in vitro, showing variable degrees of initial decline, followed by increases. As the cells reached the end of their proliferative life span in vitro, an up-regulation of these ECM genes was observed. FN increased first, followed by α_1III, and then α_1I. A similar overlapping cascade of up-regulation of these three ECM genes is observed during late fetal development and wound repair in adulthood. Thus, the proliferative phase of fibroblasts in vitro may be a diversion from the in vivo differentiation program, which is resumed as the cells approach or enter the terminal non-proliferative stage. [Supported by NIH AG-07875]

V-1018 Proliferating Cell Nuclear Antigen Expression in Aging Human Diploid Cells. C.A. Stewart and R.T. DELL'ORCO. The S.R. Noble Foundation, Ardmore, OK 73402.

Human diploid fibroblasts (HDF) lose their ability to enter S-phase and replicate DNA as they proceed through their in vitro lifespan. Proliferating cell nuclear antigen (PCNA) has been reported to be required for DNA replication. The expression of PCNA in low and high PDL cells was examined by determining mRNA and protein levels at GO, G1, G1/S, and S phases of the cell cycle by Northern and Western blot analysis. PCNA mRNA turnover at G1/S was examined by treating HDF with actinomycin D (act D) and analyzing PCNA mRNA levels at various times after treatment. Transcription of PCNA was determined by nuclear run-on reactions. The results show that in both low and high PDL HDF, PCNA mRNA levels were barely detectable at GO, increased during G1, peaked at G1/S, and declined during S. When compared to low PDL cells, high PDL cells showed 3 to 4 fold lower PCNA mRNA levels at all sample points; however, nuclear run-on analysis showed an equal amount of PCNA transcription occurring in both low and high PDL populations. The act D turnover studies indicated that PCNA mRNA has a half life of 12 hrs in both low and high PDL cells. Western analysis showed PCNA protein was present throughout the cell cycle in low and high PDL cells; but, similar to the RNA results, protein levels were 3 to 4 fold lower in high PDL cells. These results confirm and extend previous results and indicate that an age related alteration in PCNA expression occurring at the RNA processing level may play a role in the failure of high PDL cells to enter S-Phase.

V-1019 Expression of Prohibitin in Young and Old Human Diploid Fibroblasts. J.K. MCCLUNG, X.-T. Liu, L.S. Walker, R.L. King, C.A. Stewart and R.T. Dell'Orco. The Samuel Roberts Noble Foundation, Inc. Ardmore, OK 73402

The amino acid sequence of prohibitin is highly conserved between human and yeast suggesting an important role in cell regulation. Microinjection of prohibitin mRNA transcripts blocks the GO to S phase transition in human diploid fibroblasts. Since prohibitin is antiproliferative and human fibroblasts have a limited number of population doublings, the expression of prohibitin was determined in human fibroblasts of low and high population doubling levels (PDL) to establish if prohibitin expression was associated with the cessation of cellular division. Prohibitin mRNA is constitutively expressed in human cells and is cell cycle regulated. Prohibitin mRNA is expressed at low levels at GO and during S phase while the peaks are at G1 and possibly G2. The levels of mRNA expression in high PDL cells are reduced as compared to low PDL cells. However, the general pattern of expression remains about the same. Senescent human fibroblasts have the lowest level of prohibitin mRNA expression. The preliminary data suggests that there is an age relationship with prohibitin expression; however, it is opposite of what would be expected for a negative growth regulator involved with senescence.

V-1020 Effect of Human Chromosome 1 on the Proliferative Potential of Various Immortal Human Cell Lines. P.J. HENSLER[1], L.A. Annab[2], J.C. Barrett[2] and O.M. Pereira-Smith[1]. [1]Baylor College of Medicine, Houston, TX 77030 and [2]National Institute of Environmental Health Sciences, Research Triangle Park, N.C. 27709.

Normal cells in culture exhibit limited division potential which has been used as a model for cellular senescence. In contrast, tumor derived, carcinogen or virus transformed cells are capable of indefinite division (immortal). Previous fusion experiments of normal human diploid fibroblasts with immortal human cells yielded hybrids having limited life span, indicating that cellular senescence was dominant and immortality due to recessive changes. Fusions of various immortal human cell lines with each other led to the identification of four complementation groups for indefinite division. Sugawara and Barrett have implicated a role for human chromosome 1 in cellular senescence, from their studies of hybrids from fusions of normal diploid human fibroblasts and an immortal Syrian hamster cell line. To determine whether this chromosome was important in the control of human cellular senescence, we have used microcell fusion to introduce a normal chromosome 1 into immortal human cell lines representing the four complementation groups previously identified. We have found that a normal human chromosome 1 causes loss of proliferative potential of an osteosarcoma derived cell line (TE85) assigned to group C. Studies are in progress to determine effects on proliferation of cell lines assigned to the remaining three complementation groups and other cell lines assigned to complementation group C. (This work was supported by NIH Grant AG05333 and T32 AG0183.)

V-1021 Expression of genes located on human chromosome 3 in quiescent and proliferating fibroblast line WI-38. **Z. MARCSEK, E.R. Zabarovsky[1], Cs. Kiss, Z. Bori, M. Kucsera.** Dept. Cell Biol., Hung. Acad. Sci and Semmelweis Univ. Med. Sch. 1082 Budapest, Üllői út 78/A, Hungary, [1]Dept. Tumor Biol., Karolinska Inst. BOX 60 400 S-104 01 Stockholm, Sweden

WI-38 human fibroblast cell line was used for RNA isolation. Quiescence was induced by 72[hrs] serum starvation, G_0 block was released by addition 10% FCS to the cells. Chromosome 3 specific NotI linking and jumping libraries had been constructed using a new cloning and selection procedure (1). DNA for cloning was isolated from mouse-human microcell hybrid cells (2) which contain only human chromosome 3 in mouse background. Cloning of DNA fragments containing NotI restriction site was performed in lambda $EMBL_{Cos\text{-}Not}$ and lambda SK phages (3). Human DNA containig clones were selected by human-specific repetitive probe. Many of the clones have been mapped in relation to the D3F15S2 locus which is located on the short arm of chr 3. As a tumor suppressor gene, significantly lost in many human malignancies (ex. renal cell carcinoma, non-small cell mammary carcinoma, etc.), is supposedly present in this region, all CG-rich cloned DNA sequences having coding potential were selected using northern blots of total and poly(A) selected RNA from normal human placenta, RCC cell lines and tumor biopsies. Coding clones were also tested on northern blots containing RNA from cycling, G_0 arrested, and stimulated WI-38 cells. References: (1) Advances in Mol. Gen., 4:281-293, 4:295, 4:401, 1991. (2) Oncogene, 5:1207-1211, 1990. (3) Nucleic Acids Res., 17:4407, 17:4408, 1989, 18:6319-24, 1990

V-1022 COOPERATIVITY OF SV40 T ANTIGEN AND RAS IN PROGRESSIVE STAGES OF TRANSFORMATION OF HUMAN FIBROBLASTS Jill A. White[1], Stephen G. Carter[2], Harvey L. Ozer[3], and Ann L. Boyd[1] [1]Hood College, Biology Dept., Frederick, MD 21701, [2]Cell Biology Dept., GLAXO Research Lab., Research Triangle Park, NC 27709, [3]NJ Medical School, Newark, NJ 07103

The human fibroblast cell line HAL was immortalized with an origin-defective temperature-sensitive T antigen. This cell line provides an experimental system for studying the cooperativity between oncogenes. In this study, HAL cells were utilized to study the cooperativity between SV40 T antigen and the ras oncogene in the progression of transformation. These studies demonstrate that HAL cells possess characteristic growth patterns, requiring 10% serum, are anchorage dependent and express T antigen. In temperature shift experiments HAL cells grown at 35C and when shifted to 39C on days 4 and 10 begin to die immediately after shift to the non-permissive temperature. T antigen is nonfunctional in HAL cells at 39C, rendering them incapable of proliferation. To determine if ras could cooperate with SV40 T antigen to produce cells with a transformed phenotype at 35C and 39C, HAL cells were microinjected with ras DNA, with or without selectable marker, generating three cell lines: RAS-HAL, NEO-HAL, and HYGRO-HAL. These cell lines are capable of growth in reduced serum concentrations, display anchorage independence and express both T antigen and ras p21. Temperature shift experiments demonstrate that these three cell lines remain viable and do not die when shifted to the non-permissive temperature. The cell lines expressing ras are still dependent upon the normal functioning of T antigen for sustained proliferation at 39C. These results suggest that T antigen and ras cooperate in progressive stages of transformation.

V-1023 Immortalization of normal human fibroblasts by treatment with 4-nitroquinoline 1-oxide (4NQO)

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Many lines of evidence support a multistep process of carcinogenesis. Normal cells are first immortalized and then become tumorigenic. Although it is important to establish an experimental model of malignant transformation of human cells in culture, normal human cells are extremely difficult to be immortalized in culture. Thus the immortalization step is a crucial event in malignant transformation of human cells. We report here the immortalization of normal human fibroblasts by treatment with 4NQO.

Normal human fibroblasts obtained from 6-w-o embryo were intermittently treated with 1 uM 4NQO. The duration of each treatment was 1 hr. After 59 times of treatment the cells were morphologically transformed, epithelial-like, and then confirmed to be immortalized. We will present cellular and karyological characteristics of the immortalized cells at this early stage of immortal transformation.

V-1024 Expression of Simian Virus 40 Early Genetic Region in Human Endothelial Cells.

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In vitro cultivation of endothelial cells allowes the study of many complex functions of the vascular endothelium. As all primary cultures human endothelial cells exhibit a limited life span in vitro thus complicating the investigations. The early region of the genom of the simian virus 40 (SV40) is known to be involved in growth stimulation and transformation of primary cells. We have electroporated a plasmid containing the early region of SV40 into human endothelial cells isolated from umbilical veins. Cells expressing the large T antigen of SV40 were isolated and characterized. They were able to devide in a culture medium without endothelial cell growth factors and showed a prolonged lifespan compared to untransfected primary cells. The karyotype was altered. Expression of von Willebrand factor was used to characterize endothelial cell differentiation and could be detected together with expression of SV40 large T antigen.

V-1025 Fluorescent In Situ Suppression Hybridization For The Detection Of The t(11;22)(q24;q12) In Primitive Neuroectodermal tumors.

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Among the small round cell tumors, differential diagnosis is particularly difficult for their undifferentiated or primitive character. In this mixed group of tumors, only the primitive neuroectodermal tumors, which include Ewing's sarcoma, show the unique and consistent feature of the (11;22)(q24,q12) translocation, which can therefore be considered a hallmark of these neoplasias.We analyzed four PNET cell lines, one osteosarcoma cell line and eight patients by fluorescent in situ hybridization with cosmid clones 23.2 and 5.8, bracketing the t(11;22) at 11q24. We detected the t(11;22) in interphase nuclei of the four PNET cell lines and in five cases of Ewing's sarcoma in which cytogenetic analysis had not been possible. Two cases of small cell osteosarcoma and one chronic osteomyelitis were also analyzed and were both normal with respect to the t(11;22). By analyzing cell lines and small round cell tumor samples by fluorescent in situ hybridization, we established that interphase cytogenetics is a rapid alternative to chromosomal analysis for the detection of the t(11;22) and represents an invaluable tool for the differential diagnosis of small round cell tumors.

V-1026 Complementation of a DNA Repair Deficiency in Three of Four Human Tumor Cell Lines by Chromosome 4. K.K. SANFORD, F.M. Price, Y. Ning and R. Parshad. National Cancer Institute, Bethesda, MD 20892; Baylor College of Medicine, Houston, TX 77030 and Howard University, Washington DC 20059.

Human tumor cells, after x-irradiation (68R) during G_2 phase of the cell cycle, show an abnormally high frequency of persistent chromatid breaks and gaps. These result from deficient repair of the radiation-induced DNA damage. A single human chromosome 4 from normal fibroblasts was added by microcell fusion to four tumor cell lines, EJ, A1698, TE85 and HT 1080 derived from bladder carcinoma (EJ, A1698), osteosarcoma and fibrosarcoma, respectively. These tumor cell lines belong to three different complementation groups (A, C and D) with respect to cell senescence-related gene(s) (Ning *et al.*, PNAS 88, 5635-5639, 1991). In three of the four lines (EJ, A1698, TE85), addition of chromosome 4 resulted in efficient repair of the radiation-induced damage to the level in normal cells. These results suggest that chromosome 4 carries a DNA repair gene(s) that complements the repair deficiency in these three lines. Alternatively, the repair deficiency in these tumor cells may result from inaccessibility of repair enzymes to damaged sites due to abnormal chromatin conformation. If so, chromosome 4 may supply a "chromatin accessibility factor" (Hanawalt, Genome 31, 605-611, 1989).

V-1027 **Oncogene Expression By a Mullerian Tumor Cell Line. J.L. BECKER*, M. FINAN* and R.H. WIDEN^. *University of South Florida, Dept. of Ob/Gyn and ^Tampa General Hospital, Tampa, Florida, 33606.**

Ovarian cancer is the leading cause of death from gynecologic malignancy. In order to better understand factors contributing to the metastatic potential of these tumors, we have established a tumor cell line derived from a mixed mullerian tumor of the ovary. Histopathologic analysis of the fresh tumor specimen revealed a highly aneuploid cell population with a high rate of proliferative activity (S phase fraction of 14.0). The predominant cell population in the fresh specimen was represented by the undifferentiated mesodermal component, with epithelial cells present as a smaller population. Long term in vitro culture (16 months, 36 passages) has resulted in the selective growth of the epithelial cell component. These tumor cells are positive for cytokeratin, negative for CA-125 and highly aneuploid, with a DNA index of 1.5. Analysis of oncogene expression by reverse transcription followed by polymerase chain reaction (PCR) amplification demonstrated that these cells constitutively express mRNA for her2/neu, Ha-ras and Ki-ras. In addition, analysis of the her2/neu gene by differential PCR showed that these cells exhibit amplification of the her2/neu gene, relative to a single copy reference gene. The results of her2/neu and ras expression by this mullerian tumor cell line are representative of findings we have obtained with freshly isolated clinical specimens of ovarian tumor. In conclusion, this cell line represents a functional in vitro model to evaluate the effects of chemotherapeutic or hormonal treatment on gene expression by ovarian tumors.

V-1028 **Alterations in the Steady-State mRNA Levels of c-Myc and p53 in L1210 Cell Lines Resistant to Deoxyadenosine.** CORY, J.G., Long, S.D., Johnson, C.E., Carter, G.L. and Cory, A.H., East Carolina University School of Medicine, Greenville, NC 27858.

Mouse leukemia L1210 cells, selected for resistance to dAdo due to the loss of allosteric inhibition of ribonucleotide reductase by dATP, had altered steady-state levels of the mRNAs for c-myc and p53. Wild-type L1210 cells had constitutive steady-state levels of c-myc and p53. Two different dAdo-resistant cell lines (Y8, ED2) had elevated mRNA levels for c-myc; the mRNA for p53 was essentially absent. There was no amplification of the gene for c-myc in the Y8 or ED2 cells. The half-life for c-myc mRNA was the same in wild-type and the Y8 cells. Run-off experiments showed that the transcription rate (TR) for the c-myc transcript in the Y8 and ED2 cells was elevated, accounting for the increased steady-state levels of c-myc. The TR for the p53 mRNA was not decreased in the Y8 and ED2 cell lines and therefore did not account for the loss of the steady-state levels of p53 mRNA in these cells. When the Y8 and ED2 cells were treated with cycloheximide there was a selective and large increase in the steady-state level of the p53 mRNA. These data indicate that a protein with a high turnover rate is involved in the specific loss of p53 mRNA. The relationship between the changes in c-myc and p53 mRNA levels in these variant cell lines has not been established. (Supported by grants from NCI, CA42070 and the Phi Beta Psi Sorority).

V-1029 **GST-P Expression and in Vitro Promotion of Neonatal Rat Hepatocytes Initiated In Utero with DMN.** L. Testolin, L. Menapace, M. Ribecco, W. Jun and U. ARMATO, Verona School of Medicine, I-37134, Italy.

To clarify the molecular events related to the initiation and promotion stages of carcinogenesis, we used a model in which rat hepatocytes were initiated by DMN on the 18th day of pregnancy, and next cultured in vitro 3-4 days after birth. In such cultures, kept in synthetic HiWo5Ba2000 medium [1], both the expression of the placental GST isozyme and the mitogenic response to a low dose of phenobarbital (PB; 10^{-10} M) given on day 4 in vitro were investigated by combining immunocytochemistry with quantitative autoradiography: the results were compared with those gained from bona fide normal neonatal hepatocytes under similar conditions. Here we report that the exposure to DMN in utero elicited the persistent anomalous presence of GST-P in the cytosol of 60% of the postnatally cultured hepatocytes. Two-thirds of such GST-P-positive cells spontaneously cycled as compared to only one third of the GST-P-negative ones. A 24-hour exposure to PB added to a very low-calcium (0.01 M) HiWo5Ba2000 medium increased the fraction of GST-P positive hepatocytes to >75% of the total, three fifths of which actively cycled. PB also decreased both relative numbers and proliferating fraction of GST-P-negative hepatocytes. By contrast, GST-P-positive neonatal rat liver (oval) cells were quite rare in primary cultures of bona fide normal livers. Our results show that the in utero exposure to DMN induces the persistent, anomalous expression of the GST-P isozyme in a substantial portion of the hepatocyte population, the majority of which appears to be committed to spontaneous proliferation in vitro, and is probably hyperexpressing the c-myc and nuclear poly(ADP-ribose) polymerase genes (as shown by parallel studies). The data also indicate that the treatment with PB further enlarges the already conspicuous size of the GST-P-positive population, confirming the idea that initiated cells enjoy a proliferative advantage in the presence of tumor promoters. [1] F. Romano, L. Menapace, U. Armato, Carcinogenesis 9: 2147-2154 (1988).

V-1030 **Antiproliferative Activity of Side Chain Derivatives of a Unique Antiestrogen (Analog II) on MCF-7 Human Breast Cancer Cells in Culture.** L.M. Overacre, K. Avor, J. Kunchandy, and R.A. Magarian. College of Pharmacy, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Ongoing efforts to develop novel non-steroidal antiestrogens demonstrating less intrinsic estrogenicity and greater antagonism than those in use have afforded a novel series of side chain derivatives of a unique diarylcyclopropane antiestrogen, Analog II. The series was synthesized and tested for its ability to inhibit the growth-stimulating action of estradiol in the estrogen receptor positive MCF-7 human breast cancer cells in culture. In our laboratory, we have used this model to investigate the relationship between antiestrogen structure and antiproliferative activity. Cells were plated in multiwell plates at a density of 4 X 10^4 cells/well in 3 ml of media supplemented with 5% calf serum, 5mM L-glutamine, and antibiotics and were allowed to attach before treatment with the test compounds. Cell growth was measured on the sixth day using the hemocytometric trypan blue exclusion method. Analog II derivatives (K-8, K-9, K-10, & K-11) produced inhibition of the estrogen dependent MCF-7 cells over a concentration range of 10^{-9} M to 10^{-6} M. The developmental rationale, structural variants, and *in vitro* activities against the MCF-7 cell line as well as a possible mechanism of action will be discussed.

V-1031 *In Vitro* Carcinogenesis of Mammary Epithelial Cells by N-Nitroso-N-Methylurea Using Collagen Gel Culture. J.R.LaDuca and D.K.Sinha, Roswell Park Cancer Institute, Buffalo, NY 14263, USA

Chemical carcinogenesis in mammals is a lengthy process which eventually culminates in the transformed phenotype, cancer. In vitro models of chemical carcinogenesis would facilitate the study of the carcinogenic process and enable the investigator to dissect out the crucial events required for carcinogenesis to occur. As carcinogenesis is believed to be a multi-step process; initiation, promotion, and progression, a multi-step, in vitro, system has been devised in our laboratory to mimic each of these stages. We have previously shown the formation of "microtumors" in collagen gels, induced by 7,12-dimethylbenz(a)anthracene. In the present study the direct acting, water soluble mammary carcinogen, N-Nitroso-N-Methylurea(NMU) was used to induce similar "microtumors". Virgin rat mammary epithelial cells were propagated and exposed to single or multiple doses of NMU while growing as a monolayer in glass petri dishes (initiation). Cytotoxicity of this carcinogen on the exposed cells was minimal, as was determined by 3H-thymidine labeling index and by determination of percentage of binucleated cells after Cytochalasin B treatment. Initiated cells were then plated into a collagen gel culture and treated with the tumor promoter 12-O-tetradecanoyl phorbol-13-acetate(TPA). Prolonged growth in the collagen gels afforded for the progression of the transformed cells into discernible "microtumors" in the three dimensional matrix of the collagen. The morphology of these "tumors" was determined by wholemount preparation and by histological sections of the gels. Fewer, in any, such structures existed in the untreated gels.

V-1032 **Relationship between DNA Topoisomerase I Gene Expression and Sensitivity to Camptothecin in Five Human Cancer Cell Lines.** P. PEREGO, G. Capranico, G. Casati and F. Zunino. Division of Experimental Oncology B, Istituto Nazionale Tumori, 20133 Milan, Italy.

Topoisomerase I gene expression in relation to sensitivity to camptothecin (CPT) was studied in five human tumor cell lines characterized by a different response to the drug, but not selected in vitro for drug resistance. The citotoxicity of CPT was evaluated in exponentially growing IGROV-1 (ovarian carcinoma), A2780 (ovarian carcinoma), A431 (cervical carcinoma), U20S (osteosarcoma) and GBM (glioblastoma) cells using the MTT assay. IC_{50} values after 96 h of treatment for different cell lines ranged between 3.5 and 203.4 nM. The different sensitivity is not explained by the cell doubling time or cell cycle distribution, since, with the exception of GBM, these cell growth parameters are comparable. U20S and GBM cells showed low levels of topoisomerase I gene expression in comparison with IGROV-1, A2780, and A431 cells which are more sensitive to CPT. These results support the role of topoisomerase I as the primary target of CPT. High level of gene expression is related to cell sensitivity in a panel of cell lines of different histotypes, thus suggesting that determination of this parameter may be an useful indicator of chemosensitivity.

V-1033 The Long Term Viability of Microvessel Endothelial Cells Derived from Human Liposuction Fat. C.B. HU, M.T. Ma, K.E. Myers and R.C. Quijano. Baxter Healthcare Corp., Irvine, CA 92714

One of the approaches to improve small diameter prosthetic graft performance is to sod a high density of autologous endothelial cells(EC) onto the graft prior to implantation. Enzymatic method has been widely used in harvesting EC from source tissue due to the yield and the viability of the isolated cells. Among the various collagenases and mixture of collagenase with other enzymes, Crude Bacterial Collagenase(CBC) has been known to release high yield of EC. CBC contains various amount of nonspecific proteases, phospholipases, clostripain, pigments etc. The viability of the cells isolated by CBC has been a concern.
Human liposuction fat was digested with equal amount of 4mg/ml CBC buffer solution. The pellet of the collected cells was resuspended in buffer and seeded into tissue culture flasks. The cultured cells were examined under a phase contrast microscope. The cells appeared similar to the commercially available umbilical vein EC. Moreover, the cell size was studied by Coulter Multisizer and proved to be similar to the size of commercial EC. The cultured cells proliferated vitally for 4 months until passage #10. Therefore, our method with CBC could produce vital EC with high yield. The same viability of isolated cells was also observed when pure collagenase was used for the isolation.

V-1034 Lymphatic Endothelial Cell Isolation, Culture and characterization. L.V. Leak, Ernest E. Just Laboratory of Cellular Biology, College of Medicine, Howard University, Washington, DC 20059.

The culture of blood vascular endothelial cells has yielded considerable new information concerning the function and biochemical properties of these cells. However, similar studies have not been done for the lymphatic endothelium. We have isolated lymphatic endothelial cells from bovine mesenteric lymphatic vessels and grown them in medium 199 containing, endothelial cell supplement, heparin and fetal bovine serum. Plated on collagen coated petri dishes the cells grew to form a confluent monolayer with a cobblestone appearance that is characteristic of endothelial cells. Silver nitrate preparations and TEM and SEM images revealed that adjacent cell were held together with various intercellular junctions. Primary and secondary subcultures were positive for the standard endothelial marker, Factor VIII related antigens. Cells were also frozen and subsequently thawed with very good recovery of growth rates and morphology. The isolation and maintenance of lymphatic endothelial cells will allow further characterization of the lymphatic vessels and provide information that will improve our understanding of the lymphatic vascular system in the normal and pathological processes.

V-1035 Fibronectin-Mediated Elongation of Microvessels During Angiogenesis in Vitro. R.F. NICOSIA, E. Bonanno, and M.R. Smith, Department of Pathology, Medical College of Pennsylvania, Philadelphia, PA 19129.

In this study we have evaluated the effect of fibronectin on angiogenesis in serum-free collagen gel culture of rat aorta. Fibronectin is a component of the provisional matrix of developing microvessels whose role in the angiogenic process has not been fully elucidated. Because of its adhesive properties and ability to bind to other extracellular matrix molecules, fibronectin may function as a permissive substrate for the migration of endothelial cells during angiogenesis. Rat plasma fibronectin incorporated into collagen gel stimulated the elongation of newly formed microvessels in a dose-dependent manner, without significantly affecting their number. The average length of microvessels increased from 317 μm in the control to 586 μm in cultures supplemented with 300 μg/ml fibronectin. Fibronectin-treated cultures became enriched in long unbranched microvessels some of which measured up to 1500 μm in length. Microvessels longer that 600 μm increased to 39% in the presence of 300 μg/ml fibronectin as compared to 8% in the control. This phenomenon was probably due to an increased recruitment of cells since the longer microvessels of fibronectin-treated cultures contained the same number of nuclei per unit length as the controls. The synthetic peptide GRGDS, a competitive inhibitor of the binding of fibronectin to its receptor, abolished the fibronectin effect causing arrest of sprouting and fragmentation of microvessels. In contrast, GRGES, a control peptide lacking the RGD sequence had no effect. Selective stimulation of microvascular elongation is a hitherto undescribed effect of fibronectin. This novel finding supports the hypothesis that fibronectin promotes the development of microvessels during angiogenesis. (Supported by NIH HL43392).

V-1036 Role of Basic Fibroblast Growth Factor in Rat Aortic Angiogenesis. S. VILLASCHI, R.F. Nicosia and M.R. Smith. Department of Pathology, Medical College of Pennsylvania, Philadelphia, PA 19129.

The purpose of this study was to investigate the role of basic fibroblast growth factor (bFGF), a potent endothelial mitogen, during angiogenesis in serum-free collagen gel culture of rat aorta. Growth of microvessels from the aortic endothelium was markedly stimulated by exogenous bFGF. Conversely, protamine sulfate, an inhibitor of the binding of bFGF to its receptor, prevented angiogenesis in a reversible fashion. To evaluate if endogenous bFGF was released by the explants, medium conditioned by aortic rings was blotted and immunostained with anti-bFGF antibody. The medium was strongly positive for bFGF during the first 24 hours and gradually lost its immunoreactivity over time. Reinjury of the explants after ten days induced marked increase in bFGF immunoreactivity in the medium, comparable to that observed on day one, suggesting that mechanical damage was the cause of bFGF release. To immunolocalize the source of bFGF, aortic explants were frozen immediately after excision from the animal or after growth in collagen gel culture, cryosectioned and immunostained with anti-bFGF antibody by the ABC method. Negative controls included substitution of primary antibody with bovine serum albumin, an irrelevant antibody or nonimmune serum. bFGF was demonstrated in intimal endothelial cells and medial smooth muscle cells; a strongly positive reaction for bFGF was also seen in sprouting microvessels. In conclusion, our data indicate that bFGF is produced by aortic cells and may play an important role in the paracrine regulation of angiogenesis in the rat aorta model. (Supported by NIH HL43392).

V-1037 Use of a Human Microvascular Endothelial Cell Line as a Model System to Evaluate Cholesterol Uptake. E.W. ADES and J.M. PRUCKLER. Biological Products Branch, Centers for Disease Control, Atlanta, GA 30333.

CDC/EU.HMEC-1 (HMEC-1) is an immortalized human microvascular endothelial cell line which retains morphological, phenotypic and functional characteristics of normal human dermal microvascular endothelial cells. HMEC-1 cells exhibit typical cobblestone morphology, express and secrete von Willebrand's Factor, take up acetylated low-density lipoprotein and rapidly form tubes when cultured on matrigel. HMEC-1 cells express the cell adhesion molecules PECAM-1, ICAM and CD44 as well as MHC class II antigen following stimulation by gamma interferon. HMEC-1 have been passaged up to 50 times with no signs of senescence. Evaluation of HMEC-1 cells and their ability to take up cholesterol is demonstrated in a linear fashion using tritiated cholesterol. This assay should eliminate some of the problems currently limiting endothelial cell microvascular research and facilitate the study of antiatherosclerotic agents and plaque formation.

V-1038 Intracellular Calcium Response to Mechanical Shearing Force in Cultured Vascular Endothelial Cells. J. ANDO, A. Ohtsuka, R. Korenaga, and A. Kamiya*. Department.of Cardiovascular Biomechanics, *Institute of Medical Electronics, Faculty of Medicine, University of Tokyo, Tokyo113, Japan

The morphology and function of vascular endothelial cells are known to be affected by hemodynamic stresses such as blood pressure and blood flow, but it is still not known whether or not the endothelial cells actually perceive physical stimulation. Cultured endothelial cells were stimulated by mechanical shearing force, and changes in the concentration of intracellular calcium ion (Ca^{++}), one of the intracellular information transfer factors, at the time of the stimulation were studied in vitro. Cultured bovine fetal aortic endothelial cells by which Fura-2, a Ca^{++} fluorescence indicator, was taken up were abraded with a latex balloon in a specially designed system, and changes in fluorescence of Fura-2 due to this shear stimulation were determined by photometric fluorescence microscopy. Immediately after the shear stimulation, the concentration of Ca^{++} in the cells was increased and reached a peak (418.6 ± 162.9 nM, n=6) within 15 seconds after the stimulation. After the peak, the concentration was gradually restored to the resting level (53.0 ± 14.4 nM, n=6). Analysis of fluorescence images of Fura-2 revealed that the cells showed a Ca^{++} response without being injured or desquamated and that there were differences in the degree and duration of reaction among cells. This response appeared even in the air where the cells did not contact the fluid. This result suggests that the flow of fluid accompanying movement of the balloon and chemical substances in the fluid are not involved in the reaction but that pure physical force is responsible for it. These results suggest that endothelial cells have the ability to perceive physical stimulation such as shearing force and to transfer the information to the interior of the cells via changes in the intracellular Ca^{++} concentration.

V-1039 Platelet-derived Growth Factor (PDGF) Stimulates NaK-ATPase α_1 mRNA Levels in Cultured Rat Thoracic Aortic Smooth Muscle Cells. C.S.Lo, T. Tamaroglio, and J.Zhang. Department of Physiology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Increased blood vessel mass can result in an increase in peripheral resistance and there-fore can cause an elevation of blood pressure. The proliferation and growth of vascular smooth muscle cells may be regulated by PDGF-induced NaK-ATPase. The effect of PDGF(BB) on smooth muscle cell proliferation was studied. [^{3}H]-thymidine incorporation into DNA in the PDGF(BB)-treated (10 ng/ml) smooth muscle cell cultures was increased 1.2 fold, 4 fold, and 14 fold at 7 h, 12 h, and 24 h, respectively. The effect of PDGF(BB) on NaK-ATPase α_1 subunit gene expression in these cells was examined. The smooth muscle cells showed an increase in mRNA level at 30 minutes and progressively increased to a peak at 4 h after PDGF(BB) treatment (20 ng/ml). NaK-ATPase α_1 mRNA levels were also PDGF(BB) dose dependent. These findings suggest that PDGF(BB)-induced NaK-ATPase mRNA levels may contribute to vascular smooth muscle cell proliferation. (Supported by USUHS grant and American Heart Association, Maryland Affiliate, Inc. grant).

V-1040 Long-Term Replicatively-Active Cell Cultures of Normal Adult Human Hepatocytes Express Liver-Specific Function. S.M. D'AMBROSIO and R.E. Gibson-D'Ambrosio and Div. Radiobiol., Dept. Radiol., College of Medicine, The Ohio State University, Col., OH 43210.

Human liver hepatocytes were established in long-term cell culture from normal adult liver tissue. Two liver tissue specimens, excess for transplant, were obtained from the Liver Tissue Procurement and Distribution System at University of Minnesota. The tissue was cut into small pieces and shipped on ice in Hanks-BSS. Primary cultures were established within 24 hr. Cell line 1007 was established from a 22 yr old male using 0.01 % trypsin and 0.1 mM EGTA and the mechanical action of the Stomacher Lab Blender. Cell line 1014, from a 8 yr old male, used 0.5 % collagenase and the Stomacher to disaggregate cells for culture. Both cell lines were grown in mAlpha-MEM supplemented with hydrocortisone, EGF, insulin, transferrin and 10% FBS. The maximum cumulative population doubling (MCPD) was 26.9 and 90 days in culture for cell line 1007; and 40.8 MCPD and 86 days in culture for cell line 1014. Both cell lines immunohistochemically stained positive for keratin and plasma fibronectin throughout the life of the culture. Cell line 1007 was strongly positive (>80% of the cells) for albumin in early as well as in the late subpassages. On the other hand, only 15 to 30 % of cells during the life of cell line 1014, stained positive for albumin. Neither hepatic cell line produced alpha-fetoprotein or exhibited gamma glutamyl transpeptidase. To our knowledge this is the first report of the long-term culture of replicatively-active normal adult human liver hepatocytes which exhibit differentiated function in culture. This success may be due to: i) dissociation of tissue prior to shipment; ii) transport media; iii) proteolytic enzymes used for and the method of disaggregation; and iv) growth conditions. Supported by Grants from the USEPA R815612 and NIEHS RO1-ES5727.

V-1041 Establishment and Long-Term Culture of Normal Human Kidney Glomerular Epithelial Cells. R.E. GIBSON-D'AMBROSIO and S.M. D'Ambrosio. Division of Radiobiology, Department of Radiology, College of Medicine, The Ohio State University, Columbus, OH 43210

Glomerular epithelial cell cultures were established in long-term culture from human fetal kidney. The cultures were established in a mALPHA medium containing insulin, hydrocortisone, transferrin, epithelial growth factor and fetal calf serum prescreened for renal cell growth. The seven cell lines tested were able to achieve an average of 38.7 ± 14.5 CPDL and 99.4 ± 29.1 days in culture. The cell lines were characterize for cell origin and function using immunohistochemical analysis of keratin and glycoprotein production and the histochemical determination of gamma-glutamyl transpeptidase activity. Keratin was produced by the cells in all subpassages indicating their epithelial origin. Gamma-glutamyl transpeptidase activity, an indicator of proximate tubular cells, was weak and diffuse in the early cell passages and absent in all later passaged cells. The URO series of monoclonal antibodies recognizing specific glycoproteins produced by glomerular, proximate tubular, distal tubular, convoluted tubular and Henle's loop cells were used to further define the cell origin. Of this series, only the monoclonal antibody recognizing the glomerular specific basement membrane glycoprotein J143, bound to the cells at all subpassages in culture. The data presented indicate that these cultured cell, expressing glomerular specific functions, originate from the glomerular epithelium. This work was supported by the National Institute of Environmental Health Grant RO1-ES3101.

V-1042 Coculture of Normal Human Small Intestine Cells in a Rotating-Wall Vessel Culture System. T.J. GOODWIN, W.F. Schroeder*, D.A. Wolf, M.P. Moyer*, KRUG Life Sciences, 1290 Hercules Drive, Houston, TX 77058, *University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284, NASA, Johnson Space Center, Houston, TX 77058

A new low shear stress, low turbulence microcarrier culture system has been developed at NASA's Johnson Space Center (NASA/JSC) that permits large-scale three-dimensional tissue culture. Tissue culture bioreactors called Rotating-Wall Vessels (RWVs) were used in conjunction with multicellular cocultivation to develop a unique in vitro tissue modeling system. Normal small intestine (NSI) epithelium and mesenchymal cells were cocultivated on Cytodex-3 microcarriers and were initiated in two phases. NSI mesenchymal cells were inoculated into the RWV at 2 x 10^5 cells/ml and allowed to attach and proliferate for 48 to 72 hours. NSI epithelium was then added at an innoculum of 2 x 10^5 cells/ml and cultivation continued for 30 to 40 days. These cocultures attained cell numbers of 4 to 6 x 10^6 cells/ml and differentiated to form tissue-like masses of 0.4 to 0.5 cm with minimal necrosis. The masses displayed apical brush borders, differentiated epithelial cells, cellular polarity, extracellular matrix, and basal lamina. Verification of mesenchymal and epithelial cell expression was determined by immunocytochemistry and scanning electron microscopy (SEM). These data suggest the RWV affords a new tissue culture model for investigation of growth, regulatory, and differentiation processes within normal tissues.

V-1043 A Model of Neural Cell Wounding and Repair: Electrophysiological and Morphological Indices. W. GROSVENOR, A. A. Messier. and H. W. Fisher, (WG) Dept. of Zoology, URI, (HWF) Dept. of Chemistry, URI, Kingston, Rhode Island, 02881, (AAM) NSMRL, NAVSUBASE, Groton, CT 06349-5900

We have recently demonstrated the role of serine proteases and their inhibitors(serpins) upon differentiation and neuritogenesis(*In Vitro 27:100A,1991*). Recently experiments examined the bioelectric activity exhibited by these cells when the level of differentiation was modified by cell wounding. Mouse neuroblastoma cells (N,A) were cultivated in F12 with 10% Fetal Bovine Serum(FBS) and after 24 hrs were sub-cultivated into F12 with 10%FBS or serum-free with 2.5ug/ml of Insulin, Transferrin, and Selenium(ITS). Cells were incubated either undisturbed or wounded (monolayers scraped gently with a rubber policeman). Prior to wounding the cells were treated with either: 1)Phosphate buffered saline(a negative control), 2)50-100ug/ml of Protease or Trypsin(serine proteases), or 3)Either 50-100ug/ml of soybean trypsin inhibitor or Aprotinin(serpins). Standard electrophysiological measurements of resting membrane potentials on individual cells were made using a WPI Intra 767 Electrometer with 20-30 Mohm microelectrodes. Morphological indices of differentiation or neurite extension and cell growth were measured up to 72 hrs following treatment. Results showed no significant difference in resting potential(10 mV) of cells in 10%FBS or 2.5ug/ml ITS. More neurites were scored in cells in ITS or pretreated with serpins, whether wounded or not. We conclude that regulation of serine proteases and inhibitors(serpins)must be carefully controlled in neural cell repair.

V-1044 **Polyamine-Phospholipide Complex (CDM) Bloks NMDA Activated Currents and Protects Neurons to Traumatic Injuries.** A.T.GYEVAI and V.Janossy. Laboratory of Tissue Culture, Institute of Experimental Medicine Hungarian Academy of Sciences, Budapest H-1450 P.O.B. 67.

The biochemical mechanisms that underlie the capability of neurons to survive under physiological and pathological circumstances are still unknown. In our earlier work we proved evidence that CDM complex is able to support the differentiation of developing nervous tissue through the terminations of proliferation in the embryonic nervous tissue in vitro. Furthermore CDM inhibited the K-stimulated 45Ca uptake in embryonic mesencephalic cultures and depressed the intracellular content of Ca2+ measuring by Fura in adult hypothalamic cells. Considering the data mentioned above it was reasonable to assume that CDM might be a potent candidate in the blocking of the neurotoxicity causing by different excitatoric amino acids through the voltage-gated Ca channel in adult brains. To assess directly the effect of CDM on the NMDA activated voltage-gated ion channel we, have choosen two models: 1. hippocampus were taken from adult rats and were treated either with 100μM NMDA or with 100μM NMDA+2μg/ml CDM and their fine structure were investigated by EM. 2. a multielectrode culture chamber system constructed by us (Acta Biologica Hungarica 1990) was used to follow the spontaneous bioelectric activities in cultured spinal cord in the presence either of NMDA or NMDA+CDM. We found that the fine structure of with NMDA+CDM treated hippocampus revealed a normal morphological structure comparing to the hippocampus treated only with NMDA. Furthermore CDM blocks NMDA activated currents in cultures neuronal cells. These findings have led to the rational for the use of exogenous CDM in an attempt to enhanche neuron survival and to rescue neurons from injuries or induced cell death in several experimental paradigms. Consequently CDM might be considered as a potent antagonist in the neurotoxicity evoked by NMDA in the neuronal tissues.

V-1045 Isolation of Putative Mesenchymal Stem Cells From Rat Embryonic And Adult Skeletal Muscle. P.A. LUCAS, A.F. Calcutt, D. J. Mulvaney, H.E. Young, and S.S. Southerland. Department of Surgery, Mercer University School of Medicine, Macon, GA 31207.

We have demonstrated the presence of a population of putative mesenchymal stem cells in the connective tissue surrounding embryonic avian skeletal muscle. These cells differentiate into at least 5 recognizable phenotypes in culture: fibroblasts, chondrocyte, myotubes, osteoblasts, and adipocytes. We have isolated a similar population from embryonic and adult rat skeletal muscle. Cells from rat leg muscle were dissected, minced, then enzymatically digested with a collagenase-dispase solution. The dissociated cells were plated and allowed to differentiate into two recognizable populations: myotubes and mononucleated cells. The cells were then trypsinized, filtered through a 20 μm filter to remove the myotubes, and the mononucleated cells were replated. Under the effect of various inducing agents, these cells differentiated into four phenotypes: adipocytes, myotubes, chondrocytes, and osteoblasts. Pending completion of clonal analysis, we classify these cells as putative mesenchymal stem cells.

Supported by the Medical Center of Central Georgia

V-1046 Growth Factor Effects and Myo-D Expression in Cultured Balb/c and Swiss Mouse Muscle Cells. M.A.L. MALEY[1], Y.G. Fan[2], M.D. Grounds[1] and J. Rossi[2]. Department of Pathology, University of Western Australia[1] and Australian Neuromuscular Research Institute[2], Nedlands 6009, Perth, Western Australia.

Muscle precursor cell replication is exceptionally more vigorous in the regenerating muscle of crush-injured inbred Swiss mice in comparison to that of inbred Balb/c mice (1). Increased expression of basic fibroblast growth factor (bFGF) has been demonstrated in necrotic and regenerating skeletal muscle cells of MDX mice (2) (an animal model of the human Duchenne Muscular Dystrophy), which, unlike human disease, can still regenerate muscle effectively. The difference in Swiss and Balb/c muscle regeneration was further investigated by the culture of hind limb muscle from each mouse strain. The proliferative response of the cultured muscle cells to bFGF, platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) was examined quantitatively and by autoradiographic methods. The relative expression of bFGF and the presence of the myoblast marker antigen n-cam was assessed by immunofluorescent staining. There were differences in the dose sensitivity and magnitude of response to bFGF and PDGF between the strains and bFGF staining was more intense in Swiss myoblasts than in Balb/c. In situ hybridisation using DNA probes for MyoD and myogenin showed little difference in the proportion of cells expressing these myoblast differentiation genes at 5 and 10 days of culture. These results show that there are strain-related differences in growth factor cell biology which may underly the different in vivo muscle regeneration capacities of Swiss and Balb/c mice.

(1) Anderson JE, Liu L & Kardami E (1991) Dev Biol 147:96-109.
(2)Grounds MG & McGeachie JK (1989) Cell Tissue Res 255:385-391.

V-1047 ISOLATION OF PUTATIVE MESENCHYMAL STEM CELLS FROM EMBRYONIC CHICK HEART. M.L. MANCINI, R.P. Wright, P.A. Lucas, and H.E. Young. Division of Basic Science and Department of Surgery, Mercer University School of Medicine, Macon, GA 31207.

The heart, thus far, has not been considered a regenerative tissue. Previous studies have shown the presence of putative mesenchymal stem cells within 11 day embryonic chick leg muscle. These cells formed five phenotypes when treated with dexamethasone during a 30 day time period. The purpose of this experiment was to determine if a population of putative stem cells existed within the chick heart, and determine the pluripotentiality of the population. Putative stem cells were isolated as separate populations from the atria and ventricles of 11 day chick embryos by mechanical and enzymatic dissociation. The cells were sieved at 90 and 20 μm to obtain a single cell suspension, plated in differentiation medium to remove lineage-committed stem cells, and eventually cryopreserved to remove contaminating fibroblasts. Thawed cells were seeded at 2500 cells/cm^2 and cultured in the presence of 10^{-10} to 10^{-6} M dexamethasone, which stimulated the appearance of adipocytes, myofiber bundles, and fibroblasts in both ventricular and atrial stem cell populations. Sheets of myocytes were also induced in the atrial stem cell population.

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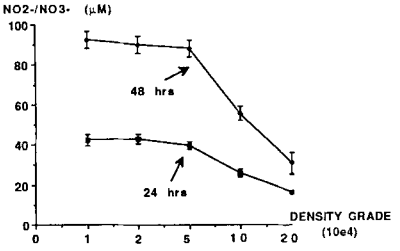
V-1048 Regulation of AFP Synthesis in the OR-HEPA Cell Line In Vitro and In Vivo. M.H. MORALES, N. Cordero and M. Urdaneta. Biology Dept., University of Puerto Rico, Río Piedras, PR. 00936

The OR-HEPA cell line, derived from C_3H mice, after many generations in culture has greatly reduced the synthesis of serum proteins such as α-fetoprotein (AFP), albumin and transferrin. In vitro treatments with ethionine or Bt_2cAMP, known gene regulators, were not able to induce the expression of these proteins, particularly AFP. The objective of this work was to analyze the tumorigenicity of clone OR-HEPA-B_3 and the effect of ethionine as an inductor of gene expression in the host environment. Cells were grown in Waymouth medium + 5% FBS and a suspension of 2.5 x 10^6 cells in 0.5 ml HBSS was injected intraperitoneally (IP). Tumors developed in all animals as an ascitic growth of unicellular and small cellular aggregates after 17 to 25 days. The ascitic fluid containing tumor and blood cells was aseptically collected. The packed tumor cells were cultured to analyze gene expression and/or transferred back into the host. To induce the synthesis of AFP in the IP growing tumor a single injection of 18 mg of ethionine was given two weeks after the tumor inoculum. 24, 48, and 72 hr later the ascitic tumor cells were plated on cover glass; the cells treated for 72 hr were positive for AFP as shown by immunofluorescence. We are currently analyzing transcription and RNA levels of tissue-specific genes. These results show the instability of the differentiated state in vitro and provide a suitable model for comparative studies of host-cell interactions which regulate gene expression.

Supported by NIH-MBRS RR08102.

V-1049 Hepatocyte (HC) Cell Density Influences the Induction of Nitric Oxide (·N=O) In Vitro. M. Di Silvio, D. Geller, V. Chough, T.R. Billiar, Z. Liu, R.L. Simmons, and A.K. NUSSLER. Department of Surgery, University of Pittsburgh, 497 Scaife Hall, Pittsburgh, PA 15261

We have recently shown that human HC, as well as rodent Kupffer cells and HC, express inducible ·N=O biosynthesis upon stimulation with LPS, IL-1, TNF, and IFNγ. Studies have shown that ·N=O has a protective role in the liver in sepsis and also exerts potent antimicrobicidal activity against hepatic malaria infection. In vitro cell density is known to influence HC function. To determine the effect of cell density on HC ·N=O synthesis, rat HC were cultured at various cell densities and exposed to a mixture of IL-1, TNF, IFNγ, and LPS for 24 and 48 hrs. Supernatants were tested for NO_2^-/NO_3^- levels as a measure of ·N=O production. At low densities ($<5x10^4$ cells/cm^2), no difference was observed in NO_2^-/NO_3^- production. In contrast, in cell densities $>5x10^4$ cells/cm^2, dramatic decreases in ·N=O biosynthesis were observed. Similar effects were seen if HC were stimulated in vivo to make ·N=O by injection of C. parvum and then placed in culture. Other parameters directly related to the induction of ·N=O production (extracellular secreted cGMP, total protein synthesis) were similarly influenced by HC density. The data presented here shows that HC ·N=O biosynthesis is dependent on cell density, which may serve important regulatory functions.



V-1050 Confocal Imaging of Cultured Human Hepatocytes Infected with Plasmodium falciparum in the SCID Mouse Model. J.B. Sacci Jr., L. Kubiak, A. Azad, J. Cottrell, K. Saccamato, R. Ascione, T. Papas and J. RESAU. University of Maryland School of Medicine, Balti. MD, 21201 and NCI Laboratory of Molecular Oncology, Ft. Detrick, MD 21702.

Plasmodium falciparum is species and tissue specific in the human host. Sporozoites, inoculated by the bite of the mosquito vector, must first invade and develop in human hepatocytes (hu-hep) to establish a clinically relevant blood infection. This species and tissue specificity has precluded extensive study of the liver, or exoerythrocytic (EE) stage of malaria.

Hu-hep isolated from immediate autopsy liver, by collagenase digestion, were cultured for several days in William's media and then transplanted under the kidney capsule of severe combined immunodeficient (SCID) mice. Human hepatocyte transplanted SCID mice (SCID hu-hep) were infected with P. falciparum, the kidneys were removed then cryosectioned and stained with several antibodies specific for parasite antigens. The slides were then evaluated using a Zeiss confocal LSM microscope. Infected hu-hep were easily identified in the sections, additionally the hepatocytes were human albumin and keratin positive when immunostained for these proteins.

Parallel cultures of human hepatocytes were infected with murine sarcoma virus in an attempt to immortalize them for subsequent studies both in the SCID model and in vitro. The hepatocytes stained positively for human albumin and keratin several months after infection. These cells are currently being evaluated for there proliferation (both in vitro and in the SCID mouse) potential by the use of hepatocyte growth factor. Additionally the msv transfected cells are being studied as a possible in vitro and in vivo (in the SCID model) replacement for primary isolate hu-heps in malaria EE stage experiments. Potential applications of these models for the study of liver cell injury, proliferation and differentiation may be quite extensive.

V-1051 An Electron Microscopic Study of Chick Iris Melanocytes in Vitro. L. SARTORI. Western New England College, Springfield, MA 01119.

Cultured chick embryo iris melanocytes were prepared for electron microscopy. The eyes from ten to eleven -day old White Leghorn chick embryos were placed in a petri dish containing Earles Balanced Salt Solution (EBSS). The eyes were bisected and the irises were peeled free from the cornea. The irises were trypsinized and cultured without feeder layers in Eagle's Minimum Essential Medium (MEM) with 10% fetal bovine serum (FBS) and antibiotics. Once the cultures were established melanocytes were lifted off the surface of the culture flask with 0.25% trypsin and gentle scraping. Sedimented cells were fixed in 2.5% glutaraldehyde, then post-fixed in 1% osmium tetroxide. This was followed by uranyl acetate staining and embedding from agar to Spurr's low viscosity resin. Sections were post stained in aqueous uranyl acetate and Reynold's lead citrate. Electron micrographs demonstrated that the cells in culture were melanocytes. Many melanosomes were present within the cytoplasm of the cells. Melanosomes varied in size and internal structure. Melanosomes were spherical or rod shaped. Some melanosomes showed an internal laminar structure.

V-1052 Testosterone Metabolism in an In Vitro Skin Model. S.R. SLIVKA, L. Landeen, and R.L. Bartel, Advance Tissue Sciences (formerly Marrow-Tech), Inc., La Jolla, CA 92037

We characterized testosterone metabolism in a three dimensional skin model consisting of keratinocytes and fibroblasts derived from neonatal foreskins. To produce a dermal model, fibroblasts were seeded onto nylon mesh and grown for 26 days until a physiological dermal-like matrix consisting of collagen, proteoglycans, and glycosaminoglycans was formed. Keratinocytes were seeded on to the dermal model and grown at the air/liquid interface until an epidermis consisting of basal, spinous cells, granular cells and stratum corneum was formed after 3-4 weeks. The model was laser cut into 2.25 cm^2 pieces and placed on 35mm tissue culture inserts with 1 ml of HEPES buffered medium on the dermal side. For comparison, foreskins and the dermal model were cut into similar size pieces and placed on the inserts. [^{3}H]Testosterone was applied to the stratum corneum side of the skin model or foreskins. To evaluate the contribution of dermal fibroblasts to the metabolism, [^{3}H]testosterone was applied to the top of the dermal model. Radiolabeled metabolites released into the medium after a 2-24 hr incubation were extracted in methylene chloride, separated by HPTLC, and quantified by autoradiography. This skin model metabolized [^{3}H] testosterone to both more polar and non-polar compounds which had the same mobility as the metabolites observed with the foreskin specimens. The appearance of non-polar compounds was earlier than the appearance of polar compounds. Fibroblasts in the dermal model converted testosterone to non-polar metabolites while an epidermis was required for conversion to the most polar metabolites. The cooperation between fibroblasts and differentiated keratinocytes in the metabolism of testosterone to both non-polar and polar metabolites is being evaluated in the skin model.

V-1053 Cultured Keratinocyte Allografts Accelerate Healing of Split Thickness Donor Wounds. R. Fratianne, I. Housini and IRWIN A. SCHAFER, Departments of Surgery, Pathology and Pediatrics, Case Western Reserve University at MetroHealth Medical Center, Cleveland, Ohio.

In this study, we compare the rates of healing induced by allografts of cultured keratinocytes applied to split thickness donor sites to healing with a standard treatment. Sheets of cultured human keratinocytes, derived from neonatal foreskins are applied to one-half of a split thickness donor site while the remainder is covered with Biobrane ^{R}. In 10 patients the average time to healing for sites covered with keratinocytes was 6.6 $\pm$ 1.96 days compared to 12.6 $\pm$ 4.32 days for control sites ($P < .002$). At day 7, the keratinocyte covered sites showed re-epithelization with the formation of a basement membrane and hemidesmosomes at the dermal-epidermal junction. Control areas were unhealed without epithelial coverage. The re-epithelized donor sites from 3 patients treated with cultured keratinocytes were reharvested. In each case, these grafts took and were equivalent to skin used from donor sites for the first time. These data indicate that keratinocyte allografts speed healing of split thickness donor sites thereby increasing the amounts of skin available for wound coverage. The increased rates of re-epithelization probably reflect the secretion of growth factors and extracellular matrix molecules by cultured cells covering the wound.

V-1054 ISOLATION OF PUTATIVE MESENCHYMAL STEM CELLS FROM EMBRYONIC CHICK SKIN. R.P. WRIGHT, M.L. Mancini. P.A. Lucas, and H.E. Young. Division of Basic Science and Department of Surgery, Mercer University School of Medicine, Macon, GA 31207.

Previous studies have shown the presence of a population of putative mesenchymal stem cells located within the leg muscle-associated connective tissue matrices of day 11 embryonic chick. Since this population of stem cells is present within the connective tissues of leg muscle they may also be present within the connective tissue matrices of the dermis. This study addressed that hypothesis. Dermal connective tissue-associated cells were isolated from day 11 embryonic chick skin by mechanical and enzymatic dissociation, sieved through 90 and 20 μm filters to derive a single cell suspension, and initially plated in differentiation medium to remove lineage-committed stem cells from the population. After the cultures reached confluence they were trypsinized, sieved, and cryopreserved to remove contaminating fibroblasts. Thawed cells were plated at 2500 cells/cm^2 and then tested for differentiation potential with dexamethasone at 10^{-10} to 10^{-6} M. Four phenotypes were noted: myotubes, adipocytes, cartilage nodules, and bone nodules. Current research is focusing on the pluripotentiality of individual cells within this population.

Supported by Rubye Ryle Smith Charitable Trust and the Medical Center of Central Georgia.

V-1055 A Molecular Analysis of Mouse Nephrogenesis in an Organ Culture System. H. YEGER, D. Forget, A. Flenniken, C. Campbell and B.R.G. Williams, Departments of Pathology and Cancer Biology, The Hospital for Sick Children, Toronto, Ontario, M5G 1X8, and Cleveland Clinic Foundation Research Institute, Cleveland, Ohio, 44195.

Embryonic mouse kidneys maintained in an vitro organ culture format continue the process of nephrogenesis. The molecular mechanisms governing the induction of metanephric mesenchyme into epithelial nephrons are still unknown although contact with ureteric bud epithelium is required to initiate the differentiation sequence. One of the genes turned on by this induction event was found as a result of the cloning of the tumor suppressor gene (WIT2) of Wilms tumor and its subsequent identification as a potential transcriptional regulator during nephrogenesis. We have modified the kidney organ culture method of Avner and colleagues (Pediat Nephrol 4: 345, 1990) and have developed a versatile organ culture system for the direct visualization of mouse nephrogenesis. Essentially, surgically resected intact embryonic gestational (days 11-15) kidneys were explanted onto transparent membrane culture inserts and grown in a defined medium supplemented with growth factors. After 24h - 7 days in culture growth of the whole organ kidneys was analyzed morphologically and immunohistochemically, and entire kidneys processed for WIT2 expression by non-isotopic in situ hybridization. The induced condensing metanephric mesenchyme and developing glomerular structures expressed increasing amounts of WIT2. No expression occurred in nephrogenic tubules or supporting stroma. These latter structures were identified with antibody to cytokeratin and with 3G5 recognizing an O-acetylated ganglioside. The organ culture results corroborate observations obtained on tissue sections and demonstrate the ability to study the nephrogenic process at the molecular level in a three-dimensional system. (Supported by the Kidney Foundation of Canada and National Cancer Institute, Canada.)

V-1056 ISOLATION OF PUTATIVE MESENCHYMAL STEM CELLS FROM THE SKELETAL MUSCLE OF EMBRYONIC CHICK AND POSTNATAL MOUSE. H.E.YOUNG and P.A.Lucas. Division of Basic Science and Department of Surgery, Mercer University School of Medicine, Macon, GA 31207.

Previous studies have shown the existence of a population of putative mesenchymal stem cells located within the connective tissue matrices surrounding embryonic chick skeletal muscle. These putative stem cells were isolated by mechanical and enzymatic dissociative procedures, differential plating to remove lineage-committed stem cells, and cryopreservation to remove contaminating fibroblasts. Treatment with various agents elicited 11 phenotypes as assessed by histochemical and immunochemical procedures. Experimentation is currently underway to assess the pluripotentiality of individual cells within this population. Since both avians and mammals share similar developmental sequences and tissue morphologies, the potential exists for stem cells to be located within the connective tissue matrices of mammals as well. To test this hypothesis, putative stem cells were isolated from postnatal mice following similar protocols. Using various inductive agents, populations of putative stem cells from postnatal mouse skeletal muscle differentiated into muscle, adipocytes, fibroblasts, cartilage, and bone.

Supported by Rubye Ryle Smith Charitable Trust and the Medical Center of Central Georgia.

V-1057 A possible role for lactate in the control of differentiation in cells secreting protein products by [o]Carmel Mothersill, [o]Sandra O' Reilly, [o]Margaret Lehane & [o]Colin Seymour, Radiation Research Centre, Dublin Institute of Technology, Kevin Street, Dublin 8, Ireland.

Lactate dehydrogenase enzymes have long been known to be involved in the control of energy metabolism. Their abnormal distribution patterns in Cancer cells have led to a link being postulated between growth of cells and high levels anaerobic metabolism. Consequent on the high anaerobic metabolism is the accumulation of high levels of lactic acid. Recently our group showed that if cultured primary human thyroid cells were supplied with culture medium containing glucose they rapidly convert this to lactate while undergoing proliferation. When the glucose was exhausted the cells began to use the lactate and to produce thyroglobulin which was secreted with the formation of characteristic follicles. When cells were fed with 14-C labelled glucose, the label accumulated in the apical membrane of the cells around the follicular lumen.

This process was thought to be unique to the thyroid but recently while culturing a hybridoma producing anti cmyc antibodies, the same phenomenom was detected. This time lactate use correlated with antibody production.

It is suggested that in cultured cells which secrete proteins lactate may act as a source either of precursors or may be involved in some way in the transport of proteins out of the cell. In an attempt to determine the pathway by which lactate is being used, cultures were fed with various inhibitors of aerobic and anaerobic metabolism and with various analogues of lactate. Inhibitors of lactate production prevented secretion of protein but inhibitors of aerobic metabolism had no effect. Secretion was unaffected if L-lactate or D-lactate were added directly. NAD+ had no effect. These facts suggest that lactate may be acting via the D-lactate - methylglyoxal pathway.

V-1058 Human Biliary Epithelial Cells For In Vitro Studies Of Biliary Atresia And Other Cholangiopathies. M.A. MORSE, S.T. Boyce, C.C. Daugherty, and M.M. Ziegler. Departments of Pediatric Surgery and Pathology, Children's Hospital, and Department of Surgery, University of Cincinnati Medical Center, Cincinnati, Ohio 45229.

Biliary atresia (BA) is the most frequent cause of persistent jaundice in infants and is most often fatal if not relieved by surgery. The etiology of BA and many other hepatobiliary diseases remains unknown and led us to investigate the normal and diseased extrahepatic biliary system in vitro. We have isolated human biliary epithelial cells (BEC) by collagenase digestion of freshly harvested gall bladders. The cells were grown in primary monolayer culture and serially passaged with maintenance of normal epithelial morphology. A serum-free culture system using a defined medium consisting of optimized nutrient medium MCDB 153 supplemented with epidermal growth factor, insulin, hydrocortisone, and bovine pituitary extract, was used for initiation of primary cultures, for frozen storage, and for serial culture. Phase contrast and transmission electron microscopy and immunofluorescent anti-cytokeratin antibody staining confirmed the epithelial nature of the cells, with 5'-nucleotidase activity documenting a specific biochemical function of the BEC. The cause of biliary atresia and other cholangiopathies is unknown, in part because of the lack of understanding of the normal signals for control of bile duct morphogenesis and growth. Thus, we have grown human BEC in primary culture using a defined medium with maintenance of both normal epithelial morphology and biochemical activity. These cultures may serve as a model system to study the cytotoxic or immunologic factors that may contribute to BA and other hepatobiliary diseases and may lead to earlier diagnosis and more effective treatment.

V-1059 Characterization of MDBK Cell Response to Epidermal Growth Factor: Mitogenesis and Receptor Binding. Walter X. Balcavage, *Anne Reilly and *Rob Moore. Terre Haute Ctr. for Med. Ed., Indiana University School of Medicine, Terre Haute, IN. and *Pitman-Moore Australia, Ltd., Princes Highway, Werribee, Vic. Australia.

To develop a reliable bioassay system for recombinant mouse Epidermal Growth Factor (EGF) we evaluated commercially available tissue culture cell lines. We found that the Maddin Darby Bovine Kidney (MDBK) cell line provided a highly useful model for studies on EGF. In characterization of MDBK mitogenesis we found that near confluent, 29-hour-synchronized cultures, pulsed with tritiated thymidine 16 hours after EGF stimulation, exhibited up to 200 fold stimulation in response to nannogram/ml levels of EGF. Synchronization was achieved by incubating near confluent cultures in culture medium lacking serum, but supplemented with RIA grade bovine serum albumin. The ED50 for mitogenesis ranged from 0.5 to 2 ng/ml in a number of trials. Mitogenesis of MDBK cells maximally stimulated by EGF was not enhanced by other growth factors including IGF-I and PDGF although each of the latter are mitogenic for MDBK cells. In EGF-radioreceptor assays we found that when 125I-EGF ligand displacement assays were performed at 5 degrees using cultures like those described above, the ligand binding was complete within 5 hours and that bound ligand was stable (zero order) for the succeeding 24 hours. At 25 and 37 degrees no stable binding state was observed. The ED50 for ligand-receptor interaction was observed to be in the same range as reported above for mitogenesis

V-1060 FACTORS INFLUENCING GRANULE CELL COLLATERAL SPROUTING IN ORGANOTYPIC EXPLANTS OF MOUSE HIPPOCAMPUS. B.W. COLTMAN, E. M. Earley and C.F. Ide. Dept. of Cell and Molecular Biology, Tulane University, New Orleans, LA 70118

Reactive mossy fiber collateral sprouting of dentate granule cell axons correlates with the development of epileptiform neural activity in rodents. Organotypic slice culture of rodent hippocampal tissue is a model system for the controlled study of sprouting in vitro. Organotypic rollertube cultures were prepared from hippocampal slices derived from postnatal day 7 (P7) mice. Timm's heavy metal staining was utilized to assay the degree of collateral sprouting by granule cell axons. Factors influencing granule cell mossy fiber collateral sprouting include: the degree of intact afferent innervation to the granule cells, the spatial position of the culture along the septo-temporal axis of the hippocampus and the length of time in vitro. Sprouting was detected one week post-culture; by two weeks, considerable sprouting was apparent; and at three weeks, intense punctate sprouting was observed in the dentate granule cell molecular layer. A septal to temporal gradient in the degree of sprouting was measured in a densitometry study including 8 hippocampi (63 viable cultures). Eighty-two percent of cultures from the septal region of the hippocampus showed heavy to medium sprouting compared to 71% from mid-hippocampal regions and 42% from temporal hippocampal regions. Afferent innervation also plays a significant role in aberrant mossy fiber sprouting. Hippocampal cultures containing an attached entorhinal cortex, the major source of afferent innervation to the granule cells, display statistically significant levels of reduced collateral sprouting in the molecular layer. Further studies are underway to determine the role additional neurotrophic factors may play in novelle circuit formation of dentate granule cell axons.

Supported by Department of Defense grant #2

V-1061 Intranuclear Actin and snRNP Aggregates Localize to Discrete, Mutually Exclusive Loci in Cultured DRG Neurons and Associated Non-Neuronal Cells. D.J. SAHLAS, K. Milankov, P.C. Park and U. DeBoni. Dept. of Physiology, Univ. of Toronto, Toronto, ONT M5S 1A8

Biochemical assays have long shown that actin and myosin are constituents of the nuclear matrix in interphase nuclei. Confocal immunocytochemistry shows that these nuclear proteins occur as distinct aggregates within interphase nuclei of intact Dorsal Root Ganglion (DRG) neurons in vitro and in vivo. Aggregates of actin occur throughout nuclei but are also associated with the nucleolar periphery, near nucleolar satellites. Use of FITC-Phalloidin shows that some nucleolar actin aggregates exist in the f-actin form. Small nuclear ribonucleoproteins (snRNPs) are necessary for pre-mRNA splicing where they contribute to the formation of active spliceosomes. Evidence exists which in part implicates nuclear actin and snRNPs in transcription processes. We now show, by confocal microscopy, that anti-snRNP antibodies mainly decorate a discontinuous "shell" of snRNPs lining the inner aspect of the nuclear envelope in DRG neurons. In contrast to neurons, snRNPs occur as aggregates throughout the nucleus in non-neuronal cells in the same cultures. In these cells, double labeling reveals that nuclear actin aggregates and snRNPs occupy adjacent but mutually exclusive loci. Histogram distributions of values derived from Nearest Neighbour analyses reveal that aggregates of actin and snRNPs are more closely associated than either actin/actin or snRNPs/snRNPs. This may indicate that nuclear actin participates in processing of nascent transcripts.

V-1062 Characterization of Enhanced Transglutaminase Catalysis in WI-38 Human Lung Fibroblasts by Ionophore A23187. M.D. MAXWELL, M.K. Patterson, Jr., P.J. Birckbichler, T.R. Norton, B.M. Fraij, G.L. Thomas and H.A. Carter. The Samuel Roberts Noble Foundation, Inc., Ardmore, OK 73402.

Confluent WI-38 cells in culture, incubated 1 hr in the presence of ionophore A23187 and Ca^{++}, showed enhanced transglutaminase activity, as indicated by intracellular protein incorporation of ^{14}C-putrescine and isopeptide crosslinks. This enhanced action was time-dependent, ionophore concentration dependent, and external Ca^{++} concentration dependent. Free cytosolic Ca^{++} was moderately increased. Few apoptotic cells were found in either controls or ionophore-treated cultures under the conditions employed, suggesting that apoptosis was minimal in this system. Measured isopeptides were enhanced several fold over controls after 1 hr incubation in the presence of ionophore and 10 mM external Ca^{++}, both in total cells and in insoluble or complex fractions of the cells. Our current concept of the characteristics of the transglutaminase product in this system will be presented.

V-1063 Localization of Cellular Transglutaminase on the Extracellular Matrix after Wounding. H.F. UPCHURCH, E. Conway, M.K. Patterson, Jr. and M.D. Maxwell. The Samuel Roberts Noble Foundation, Inc., Ardmore, OK 73402.

Tissue transglutaminase (TGase) is a calcium dependent intracellular enzyme which is widely distributed and whose biological function has not been fully determined. A significant amount of the enzyme can be detected associated with extracellular matrix (ECM) fibronectin after membrane perturbation or after addition of TGase from an exogenous source. Untreated cultures of WI-38 lung fibroblasts have never shown extracellular TGase but immunofluorescent techniques demonstrated the extracellular localization of TGase soon after puncture wounding monolayers of unfixed cells. The ECM-bound TGase remains at the wound site for many hours and is catalytically active for at least 24 hours. The binding of exogenous TGase to the ECM was used to study the characteristics of this binding process. Prior incubation of exogenous TGase with 10 mM $CaCl_2$, $MgCl_2$, $SrCl_2$ or $MnCl_2$ caused a 2-3 fold increase in binding of TGase to the matrix compared to controls. Prior incubation of exogenous TGase with GTP, GDP or ATP had very little effect on TGase binding to the ECM. Several potential treatments to release bound TGase were tried but only 2M KSCN was found effective under the conditions used. Prior treatment with 2M KSCN did not destroy the binding site since, after removal of bound TGase, more TGase would attach to the ECM. At the present time, the function of the active enzyme bound to the ECM is unknown, although its contiguous presence at the injury site suggests a possible involvement in the wound healing process.

V-1064 Involvement of C/EBP Family Proteins and NF-*k*B-like Factors in Regulation of Complement C3 Gene Expression. T.S. JUAN, [1]D.R. Wilson, and [1]G.J. Darlington. Institute for Molecular Genetics and [1]Department of Pathology, Baylor College of Medicine, Houston, TX 77030.

The third component of complement (C3) is critical in host defense in that it is involved in both the classical and alternative pathway of complement activation. The serum level of this protein is increased when the host receives inflammatory stimuli, primarily due to elevated expression in liver. This response of liver to inflammation, the acute phase response, is due to elevated level of transcription. The promoter of human C3 gene has been cloned, and by using deletion mutagenesis studies, a 58-bp region of this promoter, from -127 to -70, has been analyzed. This segment of the C3 gene promoter confers inducibility in cultured human hepatoma (Hep3B$_2$) cell line by cytokine interleukin-1 (IL-1), which is one of the major lymphokines involved in stimulation of hepatic acute phase reaction. By performing site-directed mutagenesis studies, we defined clearly two consensus sequences that mediate the inducibility by IL-1: one C/EBP conseusus sequence, and one NF-*k*B-like element. Mutation in this region causes severe reduction, often less than 10%, of basal expression, and cessation of IL-1 inducibility. Binding of protein factors in cellular extracts to these two sites were also examined in bandshift studies. Both sites are able to bind certain protein factor(s). The difference in bandshift pattern between untreated cell extract and IL-1-induced cell extract is only observed in the region that shares C/EBP consensus sequence, suggesting factor(s) binding these two sites may play different roles in regulating C3 gene expression. These data strongly suggest that both C/EBP family proteins and NF-*k*B-like factors are involved in the regulation of complement C3 gene expression during acute phase response.

V-1065 Steroid-Responsive Rat Primary Myometrial Cell Cultures for the Study of Gap Junctions. M.S. MARTY and R. Loch-Caruso. University of Michigan, Toxicology Program, Ann Arbor, MI 48109.

It is hypothesized that gap junctions allow for rapid, low-resistance propagation of depolarizing signals within the myometrium at term, thereby promoting synchronization of uterine contractions during labor. While Garfield and his colleagues demonstrated that gap junction formation in the myometrium is controlled in vivo by steroid hormones, a suitable cell culture system for study of this phenomenon has not been available. The present experiments were designed to establish a hormonally-responsive rat myometrial cell (RMC) culture system for the study of gap junctions. Initial experiments showed that culture in the presence of ß-estradiol and 10% steroid-stripped bovine calf serum (SSBCS) resulted in very low levels of estrogen receptors (ERs) in RMC cells isolated from mature pregnant rats, whereas higher ER concentrations were present in cells isolated from immature nonpregnant rats. Subsequent experiments used RMC cells isolated from immature rats. RMC cells maintained in 10% SSBCS had the lowest ER concentrations; cells in 5% SSBCS and 5% steroid-stripped fetal bovine serum (SSFBS) had intermediate ER concentrations; and cells grown in 10% SSFBS had the highest levels of ERs. RMC cells treated with 10 nM progesterone in the presence of 1 nM ß-estradiol for 6 d showed decreased ER levels, providing indirect evidence for the presence of progesterone receptors in these cultures. To determine if progesterone altered gap junctional communication, Lucifer yellow dye transfer was monitored following microinjection. RMC cultures treated with 1 nM ß-estradiol with or without 5 or 10 nM progesterone for 24 hr showed significantly decreased gap junctional communication: control cells exhibited 90% transfer, while cells treated with 5 and 10 nM progesterone showed 85% and 74% transfer, respectively. Thus, RMC cultures represent a steroid-responsive in vitro system for the study of myometrial gap junctions.

V-1066 Keratinocytes Stimulate Murine Megakaryocytopoiesis. G.D. KALMAZ, E.E. Kalmaz, and M.M. Guest. Departments of Internal Medicine, Pharmacology, and Physiology, University of Texas Medical Branch, Galveston, TX 77555.

We studied the capacity of keratinocytes (Kcs) to produce hematopoietic colony-stimulating factor(s) (CSF) to induce hematopoiesis in bone marrow by activating immature megakaryocytes (Mks). For these experiments, C_3H mice Kcs at a density of 5 x 10^4 cells/well in 0.5 ml of Dulbecco's modified Eagle's complete medium with or without 5% heat-inactivated fetal calf serum (FCS) were put into 24-well plates that were previously coated with fibronectin (500 µg/ml). In some experiments silica (100 µg/ml) was included in the media at the time of initial seeding to stimulate Kcs. As determined by trypan blue dye exclusion, viability of Kcs immediately after trypsinization was 95%. In all cases, Millicell™ (Millipore Corp., Bedford, MA) inserts seeded with 1 x 10^4 C_3H mouse bone marrow cells in 0.5 ml of medium were also placed in the wells. The cultures were then incubated in a fully humidified atmosphere of 5% CO_2 in air at 37° C for 3-7 days. At daily intervals mouse bone marrow cultures were stained for acetylcholinesterase for identification of Mks. The results showed that culture supernatants of Kcs grown in serum-free media with silica considerably stimulated Mk proliferation compared with Mk control cultures and with those cultured in the presence of 5% FCS with or without silica. The results of these investigations provide additional data on the regulation of megakaryocytopoiesis by Kc-derived CSF.

V-1067 Induction of TNFα Activity in Mice Experimentally Infected With Japanese Encephalitis Virus. G. R. Soni, K. Banerjee, R. M. Kolhapure, C. N. Dandawate and A. ROY, National Institute of Virology, Pune-411001, INDIA

Japanese encephalitis(JE) is one of the predominant viral diseases in India. Although clinical,biochemical and immunological tests are available to arrive at a fair diagnosis but like other viral diseases there is no sure enough prognostic parameter of this disease. We planned to see whether levels of cytokines do modulate as a result of virus infection which in turn can influence the pathological process and finally the prognosis of the disease state. TNFα is one of the very few cytokines which are implicated in shock and coma associated with severe parasitic and bacterial infection. We have found that in our laboratory animal model using Swiss mice that TNFα is released in peritoneal cavity and the activity peaks in 24 hours post infection and the activity in serum peaks in 3 days after infection of JE Nakayama. Using inactivated mouse brain vaccine(Nakayama) we do not find induction of such activity in intra peritoneal washings and in serum. Similarly, preliminary studies with an attenuated strain of JE(724038) do not show induction of TNFα activity in serum or in intra peritoneal fluid.

V-1068 Effect of *in vitro* infection with ureaplasma diversum on endometrial cell function. J.J. Kim*, P.A. Quinn+ and M.A. Fortier*. *Ontogénie et Reproduction, Centre de Recherche du CHUL, 2705 Blvd Laurier, Ste-Foy, QC - G1V 4G2 and +Dept. Microbiol. Hosp. Sick Childrens, Toronto, Ontario.

The presence of mycoplasma in the genital tract may be responsible for infertility in several species. However a clear evidence showing alteration of uterine receptivity in infected females is lacking. Ureaplasma diversum is known to increase infertility in the bovine. We have developed a model to study *in vitro* the effect of infection with ureplasma diversum on endometrial cell function. Coculture of endometrial epithelial or stromal cells with U.D. in presence of 10% FBS resulted in normal growth patterns of endometrial cells and U.D.. However, in absence of serum U.D. grew better in combination with epithelial than with stromal cells. This suggests that U.D. extracts essential elements from epithelial host cells *in vitro*. Since ureaplasma have phospholipase activity in their cell wall it may alter membrane composition or signal transduction in host cells. Thus we measured receptor activation and second messenger generation following infection. We have found that generation of cAMP measured in intact cells or in cell membranes was affected following infection. The effect was variable depending on the cell type, hormonal status of the cells and duration of infection. Following infection, the generation of cAMP was increased in stromal cells isolated from mid-luteal phase but not from early cycle. In epithelial cells, the effect was variable with no definite trends. During infection, we have found that cAMP generation was affected differently at different time exibiting a wave type of response corresponding possibly to growth waves of ureaplasma. Our results suggest that infection with ureaplasma diversum affects
endometrial cell function, but only under specific physiological conditions. These observations may explain individual differences in response to mycoplasma infection *in vivo*.

V-1069 Identification of a Novel Low Molecular Weight Growth-Promoting Factor Derived from Human Leukemia Cell Lines. Tao Dao, S. Nakamura, V. Holan and J. Minowada. Fujisaki Cell Center, Hayasibara Biochemical Laboratories, Inc., Fujisaki, Okayama, 702 Japan

A novel cell growth-promoting activity was identified in the culture supernatants from some leukemia cell lines. As determined by cell counting or by ^{3}H-thymidine uptake, this factor stimulated, at least 3 times the growth of T-cell leukemia cells, HPB-ALL, in low concentration (0.5%) of FCS. The factor was isolated and biochemically characterized from the supernatant of a B-cell leukemia line, BALL-1. Dialysis and FPLC on Superose 12 column chromatography indicated that the M.W. of the factor was less than 10 kDa. Growth promoting activity was resistant to heating for 5 minutes at 100 °C and to pH ranging from 2-10, but it was sensitive to trypsin and proteinase K treatment. Specific antibodies against human EGF, TGF-α, insulin and EGF receptors did not inhibit the growth-promoting activity in the BALL-1 supernatants. Furthermore, none of the known human low M.W. growth factors, such as EGF, IGF-I, IGF-II and insulin, supported the growth of HPB-ALL cells. These observations strongly suggest that the growth-promoting activity spontanously produced by some of the leukemia cell lines is mediated by a novel low molecular weight growth factor. (This work was supported in part by the Hayashibara International Cancer Research Fellowship program and the Special Coordination Funds from Science & Technology Agency of Japan).

V-1070 Growth and production of hybridoma in a lipid containing serum free medium. K. Hansen, Cell Culture, Bioscience, Novo Nordisk A/S, Niels Steensensvej 1 DK-2820 Gentofte, Denmark.

A NSO based hybridoma cell line was cultured for production of antibody to purify recombinant coagulation factor VIII. To avoid bovine IgG and high protein content, the medium had to be free of serum and albumin. This cell line will only grow and produce well in medium containing adequate lipid supplement. Intralipid made for clinical use supported growth and production of the cells

Intralipid is a microemulsion made from fractionated soy lipid and lecithin. In a repeated batch and upscaling in spinners the cells reached a max. of 1.8 x 10^6 cells/ml and 100 mg/l of antibody before subculturing. In the final 1.3 l NBS bioreactor batch the antibody concentration reached 215 mg/l medium. About half the antibody appeared in the medium during the final growth phase and the medium was harvested when less than 10% of the cells were alive.

V-1071 **Efficient Production of Recombinant α-Amidating Enzyme in a Stirred Tank Bioreactor.** D. JACKSON-MATTHEWS*, C. Pray and K. Piparo. Unigene Laboratories, Inc., Fairfield, NJ, 07004.

CHO cells engineered to secrete the 75 kDa rat α-amidating enzyme (α-AE) provide a catalytic reagent for use in the manufacture of biologically active peptide hormones that require C-terminal amidation. Suspension growth technology has been investigated as a means of large-scale production of recombinant α-AE. Cell specific productivity was 3.0 μg α-AE/10^6 cells/day for static cultures of suspension-adapted or anchorage-dependent cells. Spinner flasks were used to examine parameters of CHO cell and serum-free medium performance: despite attaining only modest densities, cells secreted 5.0 μg/10^6 cells/day for an overall yield of 2.5 mg/L of medium consumed. Preliminary scale-up was performed with a 4L stirred tank bioreactor in which temperature, pH, dissolved oxygen and agitation rate were monitored and controlled. Batch mode operation yielded 4.0 μg α-AE/10^6 cells/day, 4.6 mg/L of medium consumed and a production rate of 1.7 mg/day. For fed-batch mode, results were 3.3 μg/10^6 cells/day, 3.6 mg/L and 2.0 mg/day, respectively. Cell densities remained $< 10^6$/ml. In an initial run at the 10L bioreactor scale, an external perfusion loop with hollow fiber cartridge was employed. Cell density increased to 3x10^6/ml, and 1.5 mg α-AE/L was produced at a rate of 6.6 mg/day. These studies demonstrate that suspension technology affords an efficient and convenient means of large-scale production of α-AE.

V-1072 **Gram Quantity Monoclonal Antibody Production in Continuous Bench-Scale Bioreactor Culture.** **Wells Magargal and Aaron Abramovitz.** Lederle-Praxis Biologicals, Pearl River, N.Y. 10965

Production of gram quantities of MoAb has been achieved in a perfused bench-scale stirred tank bioreactor. Cells are recirculated through two parallel hollow fiber cartridges. Clarified culture medium containing the MoAb is harvested through one cartridge while fresh medium is added through the other. The harvest and feed flows are periodically reversed to reduce clogging. Cells are initially grown in medium containing fetal bovine serum. The serum content is gradually reduced by perfusion until the culture is essentially serum free. Cell densities are maintained at 5-10x10^6/ml and perfusion rates are adjusted to maintain nutrients and metabolites at appropriate levels. Dissolved oxygen and pH are controlled by a 4 gas controller and addition of bicarbonate solution. The culture described here was maintained for 31 days. MoAb concentrations ranged from 100 μg/ml early in the culture to a peak of 500 μg/ml. MoAb in the harvest averaged over 200 μg/ml with a total harvest of almost 20 g. Productivity was 4-5 g/wk and the specific productivity for this culture was 35-40 ng/cell/day. MoAb isotype did not change and electrophoretic and isoelectric profiles of the MoAb as determined by western analysis were virtually identical throughout the culture period. These studies show that gram quantities of MoAb can be produced in bench-scale continuous bioreactor culture and that the MoAb produced remains biochemically and immunochemically stable.

V-1073 Three-Dimensional Modeling of T-24 Human Bladder Carcinoma Cell Line: A New Simulated Microgravity Vessel. T.L. PREWETT, T.J. Goodwin, R.P. Schwarz, G.F. Spaulding, KRUG Life Sciences, 1290 Hercules Drive, Houston, TX 77058, NASA, Johnson Space Center, Houston, TX 77058

This abstract presents a new device and method for the tissue culture of high oxygen requiring cell lines and primary tissues. The High Aspect Rotating-Wall Vessel (HARV) described has successfully cultured T-24, a human transitional epithelial bladder cell line, on Cytodex-3 microcarriers in three-dimensional cellular aggregates up to 0.5 cm in diameter. The HARV is a horizontally rotated tissue culture vessel with a large surface-area-to-volume ratio silicone membrane oxygenator. This design augments the previously disclosed Rotating-Wall Vessel (RWV) termed the Slow-Turning Lateral Vessel (STLV) in a low turbulence, low shear, cell growing environment by increasing the oxygen delivery capability. These studies detail a comparison in the metabolic glucose performance as a function of cell growth of T-24 in both the HARV and the STLV versus standard T-flask culture. The HARV has demonstrated the potential to culture a variety of cell types including normal and neoplastic anchorage-dependant and suspension cells. These cell types include hybridomas, human lymphocytes, and human glioma spheroids.

V-1074 **Prescreening of Potential Chemopreventive Agents using Biomarkers of Transformation.** S. Sheela, J.D. Stutzman, E. Korytynski, K.R. Garris and V.E. Steele*. ManTech Environmental Technology, Inc., Research Triangle Park, NC 27709 and *Division of the National Cancer Institute, Bethesda, MD 20892.

A high priority in cancer research is to search and identify biological markers that exist in causal pathways to cancer because of their relevance to cancer prevention. A number of *in vitro* screening assays has been developed to evaluate the chemopreventive potential of 169 compounds using a biochemical parameter known to be associated with initiation, promotion, or both processes of carcinogenesis. An assay based on the inhibition of promoter-induced tyrosine kinase activity was done in the particulate fraction of human leukemic cells after exposure to 12-O-tetradecanoyl phorbol-13-acetate (TPA, 100nM) alone or in the presence of five doses of a chemopreventive agent for 24 hours. Of 169 compounds screened 117 compounds were positive in this system. Inhibition of ornithine decarboxylase activity (ODC) was determined in cell free extracts of rat tracheal epithelial cells (2C5) after the cells were exposed to TPA (0.5μM) alone or in the presence of a chemopreventive agent for five hours. This assay system identified 117 compounds as ODC inhibitors from 152 tested. Inhibition of carcinogen-DNA binding, another screening assay involves the exposure of immortalized human bronchial epithelial cells (BEAS-2B) to ^{3}H-benzo[a]pyrene (B[a]P) for four hours after they were pretreated with five doses of a CPA and determining the bound ^{3}H-B[a]P after DNA extraction. Forty-seven out of 100 compounds were found to be positive in this assay. Testing 152 compounds using inhibition of poly(ADP-ribose)polymerase (PADPRP) activity was done in primary human skin fibroblasts after the cells were exposed to a carcinogen, propane sultone. Confluent cultures were exposed to propane sultone (41nM) alone or in the presence of various doses of CPA for 18 hours and cell free extracts were assayed for PADPRP activity using ^{32}P-NAD as substrate. Seventy-seven of 100 compounds were positive in this assay. Some of the compounds which were highly positive in all the assays are bismuthiol, carnosine, butylated hydroxyanisole, centrophenoxine, difluromethyl ornithine, ellagic acid, esculetin, etoperidone, 18-β-glycyrrhetinic acid, indole-3-carbinol, indomethacin, piroxicam, suramin, thiolutin, tocopherol succinate PEG 1000, ascorbic acid, and vitamin K_3. Results from these assays indicate that a large number of compounds can be screened in a rapid and reliable manner and that compounds which are positive in all the assays have a varied mechanism of action inhibiting a number of biochemical endpoints associated with transformation. This work was supported by NCI Contract N01-CN-95172-03.

V-1075 Use of Expanded Surface Roller Bottles for Production of Marek's Disease Virus Vaccine. J.R. MOLDENHAUER, T.H. Robinson, L. Ryan, and B. Nordgren, Solvay Animal Health, Inc., Mendota Heights, MN 55120.

Commercially-supplied polyester roller bottles* with surface areas of approximately 2.3 times (2125 cm^2) and 4.4 times (4100 cm^2) greater than conventional glass roller bottles (940 cm^2) were evaluated for efficiency of cell and virus production. Conventional roller bottle technology for culture of cells and production of viruses is both labor and space intensive. Improvements in the current roller bottle technology have been achieved through expansion of surface area available for cell attachment and growth. Surface area has been increased by use of a pleated design with channels for removal of culture fluids. Primary chicken embryo fibroblasts (CEF) are used as the cell substrate for propagation of Marek's Disease Virus (chicken herpesvirus). Virus-infected viable cells are harvested and formulated to produce a live vaccine product. Twenty experimental runs resulted in CEF yields that increased with surface area. For example, use of the 4100 cm^2 bottle resulted in CEF yields ranging from 2.9 times to 5.2 times greater than conventional glass bottles. Virus plaque-forming units produced exceeded the minimum virus titer requirement. This improved roller bottle technology was successfully implemented for production of this MDV vaccine. Expanded surface roller bottles provided a means of increasing product yields per bottle by virtue of increased surface area. *Provided by In Vitro Scientific Products, Inc., Ventura, CA 93003.

V-1076 In Vitro Studies of Endogenous Adenine Nucleotide Contents in Relation to Sperm Motility and Acrosomal Protease Activity. J.S. CHEN, M.G. Menesini Chen, C. Sensini, M. Bari, M. Barbetti, B. Baccetti. CSCG del CNR, Siena 53100, ITALY.

Flagellar sperm motility is known to play a role in the gamete interaction both in vivo and in vitro. The motility requires the supplement of energy. Even if there are many factors involved in the initiation and the maintenance of sperm motion such as Ca++ and ATP ect., it is still uncertain about the role of endogenous adenine nucleotides (ATP, ADP and AMP) in support of motility. In order to see the disposition of these nucleotides in relation with the motility and the acrosomal protease activity, we have put washed 3x boar epididymal spermatozoa in medium (Tris Buffer Saline, pH 7.2) deprived of any exogenous energy sources with and without ascorbic acid. Falcon dishes placed in desiccator and saturated with air or nitrogen were kept at 37°C in an incubator for up to 24 hrs. In general the sperm motion, twitching, (Moor et al, 1986) was observed in control dishes either maintained in air or in nitrogen up to 6 hrs, while the sperm cultured in the presence of ascorbic acid were immotile. As to the adenine nucleotide contents (ATP + ADP+AMP) of samples maintained in vitro, the initial value is 0.91 mM /40x10^6 sperms, but it decreases rapidly to trace level within 6 hrs in both cases. The data also show that in the presence of ascorbic acid the conversion of proacrosine to acrosine is partially inhibited. Thus the present culture system which provides the motile and immotile sperms in vitro could be a model study for the better understanding of sperm energy requirements.

V-1077 Evaluation of a Group of Petrochemicals Using Clonetics' Neutral Red Bioassay to Predict Irritancy. R. BARSTAD, J. Janus, J. Lauten, N. Accomando, A. Triana. Clonetics Corporation, 9620 Chesapeake Drive, San Diego, California 92123-1324.

The normal human keratinocyte based NeutralRed Bioassay (NRB), adapted from the procedure of Borenfruend and Puerner, is one of several in vitro alternative cytotoxicity tests. We have previously demonstrated that this assay can predict the irritancy of several different classes of compounds (personal care products, shampoos, perfumes, insoluble talcs, alcohols, metal salts, burn ointments). The current study examines the irritation potential prediction capability of the NRB with a group of 52 petrochemicals for which Draize data were available (supplied by Mobil Oil Corporation).

The water insoluble compounds were eluted overnight into Keratinocyte Growth Medium (KGM) before application to the cultured keratinocytes and testing in the NRB.

* The neutral red uptake (NRU) assay correctly identified (irritating or non-irritating) 48 of the 52 compounds.
* Three of the compounds were classified as irritating by NRU but had Draize scores in the non-irritating range (false positive).
* One compound was classified as non-irritating by NRU but had a Draize score in the irritating range (false negative).

Eight compounds, which were previously tested in the NRU, were tested in the neutral red release assay (NRR). Tested were three false positives, one false negative, three true positives, and one true negative.

* The three false positives were correctly identified as negative.
* The three true positives were correctly identified as positive.
* The true negative tested correctly as negative.
* The false negative tested incorrectly negative.

Further studies indicated the reason for the false negative was failure of the toxic constituent(s) to elute into KGM.

V-1078 Update on the Incidence of Mycoplasma Contamination Detected in Cell Lines and Their Products. J. LUCZAK, S.A. Knower, M.S., Cox, J. Dubose Jr., and J.W. Harbell*. Microbiological Associates, Inc., Rockville, MD 20850

Mycoplasma contamination of cell substrates and the resulting products continues to be a serious concern to basic research and to the biotechnology industry. These organisms can be introduced through a variety of routes including the initial isolation, culture reagents, and/or cross contamination within the laboratory or production facility. Use of antibiotics in routine culture has also served to mask but not remove mycoplasma contamination. This study was conducted from 1985 to 1992 and all samples were tested using three culture methods; direct inoculation onto two kinds of agar (modified Hayflick's media), inoculation into broth with three subcultures, and inoculation into Vero cell cultures with subsequent Hoechst staining for DNA. Two classes of assays were performed, research level testing on cells and products not intended for regulatory submission and regulatory level testing intended for regulatory submissions. Under the research level testing program, 2483 cell lines were examined with 343 positives (13.8%), 174 products were tested with 6 positives (3.4%). The predominant organisms isolated were M. arginini, M. hyorhinis, or M. fermentans. Under the regulatory testing program, 741 cell lines were tested with 31 positives (4.2%) and 2067 samples of product (all stages) were tested with 19 positive (0.9%). The predominant organisms isolated were M. hyorhinis, M. arginini, M. orale, or M. salivarium. Of particular note has been the increase in the incidence of contaminated cell lines and unprocessed bulk product submitted for regulatory level testing during the past year. These data show the importance of testing cell cultures early in their development cycle, controlling all portals of entry, and continuing to test cell substrates and their products routinely throughout their development and production.

V-1079 Major Metabolic Changes Accompany Transfection and Selection for High Level Expression of Recombinant Genes. S. GOULD, D. DiStefano, G. Cuca, D. Robinson, and M. Silberklang. Merck Sharp and Dohme Research Laboratories, P. O. Box 2000, Rahway, NJ 07065

The use of mammalian expression vectors bearing amplifiable marker genes has become widely accepted as a means to high level expression of recombinant genes. Typically, the resulting recombinant clones are grown in the same basal medium as the parental cell line. We observed that, in many cases, high level gene expression is accompanied by altered growth characteristics and nutrient consumption so that modification of the basal medium nutrient composition is beneficial. We report the effect of transfection, amplification and single-cell subcloning on glucose uptake, lactate production and amino acid use rates of NS/0 cells bearing Glutamine Synthetase (GS)-based expression vectors. Untransfected NS/0 cells rapidly deplete only glucose and glutamine from the medium. The use of most other amino acids is almost negligible. Clones transfected with the GS gene consume substantially more glutamate and asparagine (an ammonia source) and significantly more of several other amino acids. These clonal lines use glucose at one quarter the rate of the untransfected cells, release glutamine into the medium, and do not produce ammonia. Differences between untransfected cells and cells overproducing recombinant antibody after gene amplification via the GS system indicate that glucose may not be the major energy source for the cell lines expressing high levels of recombinant antibody and that fine-tuning of the basal medium amino acid composition is necessary for each clonal line. Since high initial concentrations of some amino acids are toxic to the cells, we developed a serum-free fed-batch process based on balancing amino acid feed rates to consumption rates. This process has yielded a final recombinant antibody titer of nearly 1 gram per liter.

V-1080 Correlation of Bacteriophage and Endotoxin Results in Animal Serum Products Jane D. Keathley
JRH Biosciences, Inc., Lenexa, Kansas 66215

Levels of endotoxin and bacteriophage have historically been used as indicators of the quality of animal serum collection. Because both endotoxin and bacteriophage indicate the presence of gram negative bacteria during the collection process, it has been assumed by some that levels of the two correlate. To test this assumption, sixty lots of serum, including 40 lots of fetal bovine serum (FBS) and 20 lots of bovine calf and donor bovine calf sera, were evaluated for endotoxin and bacteriophage using standard assay methods. Endotoxin levels were assayed by the chromogenic LAL method. Bacteriophage levels were determined by an agar overlay assay. Presence of phage (i.e. >2PFU/ml) correlated with increased levels of endotoxin (>1EU/ml) in only 36 of 60 lots (60%). In 24 of 60 lots (40%), including 15 of 40 (38%) lots of FBS, either phage or endotoxin levels were elevated, but not both. Higher levels of phage (>10 PFU/ml) correlated with higher endotoxin levels (>10 EU/ml) in 45 of 60 lots, with 15 lots (25%) showing no correlation. Data show that correlation between endotoxin and bacteriophage is inconsistent and the presence of one should not be used to predict the presence of the other. Reasons for the discrepancies are discussed.

V-1081 The Effect of Serum Filtration on Cellular Attachment and Growth.
M. MAZUR-MELNYK and A.L. SYMINGTON. Connaught Labs. Ltd., 1755 Steeles Ave. W., Willowdale, Ontario, Canada M2R 3T4.

Bovine serum is used as the growth stimulator for most tissue culture applications. Concern over the presence of adventitious and exogenous agents has led to the filtration of serum through increasingly smaller pore sizes. We examined the effect of filtration on cellular attachment and long term growth of the human diploid cell lines MRC-5 and WI-38. Serum that had been filtered through a 0.2 μm filter was further filtered in the laboratory through a 0.1 μm filter. Serum was also heat-inactivated before and after filtration. Cellular attachment was adversely affected by filtering the sera through a 0.1 μm filter. Long term cultures (> 1 week) were maintained longer when supplemented with 0.2 μm filtered sera as compared to 0.1 μm filtered sera. Heat-inactivation of sera before filtration led to 30% less growth than heat-inactivation following filtration. This is of significance to both filtering raw sera and filter sterilizing media containing serum.

V-1082 Cell Culture Optimization and Cell Bank Establishment of the Human Cutaneous T Cell Lymphoma Cell Line HuT 78.
M. MAZUR-MELNYK and M. LACATUS. Connaught Labs. Ltd., 1755 Steeles Ave. W., Willowdale, Ontario, Canada, M2R 3T4.

Current applications of lymphocyte cell lines are for HIV related studies and cancer immunotherapy. During cell line isolation difficulties are readily encountered in the inability to expand the culture. Also depending on selective culture pressures, cell line characterization and stability may vary with prolonged growth. We have studied the growth, morphology, and population stability of the human cutaneous T cell lymphoma cell line HuT 78 (ATCC TIB 161). We have established cell banks at various culture intervals, and optimized the growth parameters for this cell line. In the initial cultures, 2 different types of cell morphologies were observed. However, with time and culture environment we were able to drive the culture to either one or the other morphology type. The influencing factors were different serum treatments and medium glucose concentrations. Karyology analysis was also conducted in order to confirm genetic stability.

V-1083 Large-scale Serial Subcultivation of MRC-5 Cells on Microcarriers.
M. MAZUR-MELNYK, R. Warren, M. Krapez, H. Cho, and D. Alkema. Connaught Labs. Ltd., 1755 Steeles Ave. W., Willowdale, Ontario, Canada, M2R 3T4.

Microcarrier culture of anchorage-dependent mammalian cells is widely employed for the production of various biologicals. Heteroploid cells originally were introduced as a substrate for human vaccine production due to scale-up difficulties encountered with human diploid fibroblast cultivation. Subcultivation of diploid fibroblasts on microcarrier beads is considered to be the limiting factor in the industrial scale-up. We report that we have successfully serially propagated the human diploid fibroblast cell line MRC-5, on microcarrier beads, up to the 1000 L bioreactor scale, for the production of inactivated polio vaccine. We were able to achieve on average more than 96% viability, 80% cell transferability, and 80% cell reattachment. The viral yields were found to be unaffected. The above transfer process is not only efficient, but also economically favourable.

V-1084 Towards an improved control of the environment for animal cells.
J.M.Coco Martin, D.E.Martens, C.A.M.van der Velden-de Groot, R.C.Dorresteijn, B.Romein and E.C.BEUVERY. Nat.Inst.for Public Health and Environmental Protection (RIVM), P.O.Box 1, 3720 BA Bilthoven, The Netherlands.

Animal cells are widely used to produce therapeutic proteins (e.g. human interferon-β1, EPO, t-PA, MAbs, etc.). Computerized cultivation systems and data from relevant on- and off-line analyses can be used for an improvement of the control of the environment of animal cells. Such a control is an important tool to increase the consistency of production.
Data have been collected during the cultivation of hybridoma cell line MN12 in continuous homogeneous culture systems. An ADI 1000 has been used as computerized measurement- and control system. The process parameters pH, temperature, stirrer speed, dissolved oxygen (DO), various gass flows, two controller outputs and the consumption of base have been measured and/or controlled. The amount of oxygen supplied to the headspace of the bioreactor has been determined continuously using a mass spectrometer. In case of a constant oxygen transfer coefficient and a constant DO, this amount equals the oxygen demand of the cells. In addition, the consumption rates of glucose and glutamine have been determined.
During the culture the DO was reduced and a change in metabolism was observed. This change in metabolism was confirmed by the glucose, glutamine and metabolite levels. The change was also reflected in the appearance of a cell population with a higher membrane IgG content, as determined by flow cytometry. The change in metabolism affected also the glycosylation pattern of the MAb. ELISAs and isoelectric focusing have been performed to characterize the amount and integrity of the produced MAb.
These data will be compared with those of the computerized cultivation system and an approach for an improved control will be elucidated.

V-1085

Optimal Cryopreservation of a Human Skin Equivalent can be Determined with the Multiple Endpoint Assay.
L. S. RHOADS, J. G. Baust and R. G. Van Buskirk.
Dept. Biological Sciences, SUNY-Binghamton, Binghamton NY 13902.

The human skin equivalent (HSE) serves as an in vitro system to examine mechanisms for differentiation, transdermal transport and toxic events. The inability to transport and store the HSE for extended periods necessitated the development of an optimal cryopreservation protocol for a human skin equivalent. The mechanisms of damage to the epidermis during freezing and the use of novel cryoprotective agents were examined through the use of a fluorescent multiple endpoint assay. The HSE were labelled with a variety of indicator fluorochromes that monitor plasma membrane integrity, lysosomal integrity, epidermal permeability, glutathione levels and free radical accumulation. These fluorescent probes were quantified in situ in the CytoFluor 2300 (Millipore, Bedford MA). Electron microscopy provided correlative information on the ultrastructural damage to the HSE. The data indicated that both plasma membrane integrity and epidermal permeability are susceptible to freezing damage. The HSE is designed for a multiple endpoint assay and information using fluorescent indicators has revealed mechanisms for optimal cryopreservation. Cryopreservation of the HSE is a critical component to the acceptance of this assay system in the area of in vitro toxicology.

V-1086

Plating Embryonic Chick Mesenchymal Cells In Serum-free, Defined Media. S.S. SOUTHERLAND, H.E. Young, and P.A. Lucas. Department of Surgery, Mercer University School of Medicine, Macon, GA 31207

Previously, cell cultures, including stage 24 embryonic chick limb bud cells, have been plated in serum-containing media to ensure cell attachment. We investigated the use of serum versus a serum-free chemically defined media (DM) to support cell attachment and viability. Cells were enzymatically digested from the limb buds, then plated in either serum-containing media or DM. Cells plated in DM attached to the culture dish and had DNA contents higher than serum on Day 1. However, on Day 2 the serum-plated cultures had higher DNA than the DM cultures. However, incubating the cells in serum-containing media for 1½ hours, then diluting and plating in DM resulted in DNA contents on Day 2 identical to plating in serum-containing media. This data indicates it is possible to severely restrict the use of serum without sacrificing cell attachment. The culture conditions can be more rigorously defined by the use of a chemically-defined media and allows the addition of exogenous factors to begin immediately while reducing interactions of these factors with serum.

Supported by the Medcen Foundation

V-1087

Development and Characterization of a Cell Line Derived From a Diploid Frog, Xenopus laevis, Homozygous for All Loci. E. M. EARLEY, D. C. Reinschmidt, and R. Tompkins.
Department of Cell and Molecular Biology, Tulane University, New Orleans, LA 70118

Homozygous diploid animals offer valuable cellular material for genetic and for molecular biological analysis. One such animal developed and cloned in our laboratory, HD #1, has been widely used for such purposes. Gynogenetic reproduction of established homozygous diploid animals is difficult. Therefore, in order to obtain larger quantities of cells, we established cell cultures from muscle tissue of a third clonal generation member at transformation (stage 59 of the Nieuwkoop and Faber stage series). The cultures were initiated from finely minced muscle tissue on medium supplemented with 10% fetal bovine serum and grown at 25°C. After the initial lag in growth, the cells were subcultured at a 1:2 ratio. Subsequently, the split ratio increased to 1:6 at passage 39 (p39) and 1:10 at p60. Cytogenetic analysis at p39 showed 50% diploidy. The 50% aneuploid cells were hypodiploid with 33 chromosomes. The most striking abnormality was that one of the two distinctive nucleolar organizers has translocated to the largest metacentric chromosome. The chromosome changes may have permitted the increased growth rate of these cells. All cultures continued to have 10-20% typical multinucleated muscle cells. Our results demonstrate the efficacy of the in vitro system for obtaining large quantities of genetically defined cells.
Supported by a grant from the Department of Defence.

V-1088

DNA Fingerprinting : a powerful tool for the differentiation of cell lines. G.N. STACEY, B.J. Bolton and A. Doyle.
European Collection of Animal Cell Cultures PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 0JG, UK

Multilocus DNA fingerprinting is applicable to a wide range of species and is increasingly being used for the analysis of cell cultures in research and biotechnology. Fingerprinting offers many advantages in comparison with standard methods of cell authentication. In routine quality control at the ECACC the Alex Jeffreys probes 33.6 and 33.15 have been used to fingerprint cell lines from a wide range of species.
Fingerprint profiles in long-term and large scale cultures of human B lymphoblastoid cells have been studied as well as the effect of Epstein Barr transformation. The differentiation of murine hybridomas is an important aspect of this work. The routine fingerprint method used was capable of differentiating hybridoma clones from the same fusion experiment. The influence of growth phase and cellular differentiation on DNA fingerprints has also been studied. These studies address important questions about the use of DNA fingerprinting for cell lines and provide useful data on the use of this powerful and widely applicable method.

V-1089 Development of Procedures for the Optimization of Electrofusion Protocols. R. HELLER*[1], M. Jaroszeski[2], and R. Gilbert[2], Univ. of So. FL, Dept. of Surg.[1], Col. of Eng.[2], 12901 N. 30th St, Tampa, FL 33612.

Cell-tissue electrofusion is an electromechanical process that can be employed to transfer specific membrane surface components from individual cells to intact tissue. Potential applications include: site specific delivery systems; improved surgical procedures; and establishment of unique animal models. The development of these applications depends on the ability to optimize electrofusion procedures. Therefore, this study was initiated to establish the protocols necessary to optimize the fusion process. These procedures were worked out first for cell-cell electrofusion (CCE). The simplex optimization method was applied to a cell-cell system composed of human HL60 cells and murine WEHI-3 cells. To run the simplex a method for quantifying successful fusion was required. This procedure was established as follows. The cells are centrifuged onto Zeta-Probe discs. The discs containing the cells are placed in the fusion chamber and fused under various conditions as directed by the simplex routine. Following exposure to the electrical fields, the disc is removed from the chamber and the cells are incubated for an appropriate interval, fixed with 1% paraformaldehyde and then exposed to a phycoerythrin conjugated anti-human-leu-M9 antibody and a fluorescein conjugated anti-mouse H-2K^d antibody. The sample is then analyzed by fluorescent activated cell scanning. Fused cells are positive for both colors and the percent fused is calculated. This methodology is now being utilized to optimize the electrofusion process.

V-1090 Fusion from Within: Light Microscopic and SEM Observations of LLC-RK1 Cell Monolayers Infected with Equine Herpesviruses. E.E. BRAKO, L. Phaire-Washington and L. Brako. Biology Department, Winona State University, Winona, MN 55987 and Carver Research Foundation, Tuskegee, AL 36088.

The morphological events central to herpesvirus-induced syncytia formation are not clearly defined. This study explores the events in LLC-RK1 cell monolayers exposed to equine herpesvirus strains KyD (EHV-1) and LK (EHV-2). At 4h post inoculation (hpi) with EHV-1, loss of cellular architecture accompanied focal vacuolation and interdigitation between adjacent cells. At 6 hpi an amorphous material, presumed to be depolymerized cytoskeletal proteins, was observed in some cells. The material seemed to form an intercellular bridge or seal at regions where outlines of adjacent cells appeared discontinuous, and its presence in cell nuclei and the cytoplasm rendered nucleoli less visible. The precise nature of the amorphous material and its role in cell fusion require further investigation. Syncytia with 50 or more nuclei, and degenerating polykaryocytes, characterized by pycnotic nuclei and cytoplasmic granulation, were seen at 24 hpi. Each syncytium, viewed by scanning electron microscopy, appeared as a broad and flat sheet structure with surface microvilli and an extracellular material, and some syncytia spanned a distance of more than 500 micrometers. LLC-RK1 monolayers infected with EHV-2 displayed morphological features similar to those caused by EHV-1, except for a 24h delay in appearance of the changes. The study provides novel morphological data and insights on herpesvirus-induced cell fusion.

V-1091 Cells on rotating fibers for fast reactions. R.A. CLYDE, Clyde Engineering, POB 740644, New Orleans, Louisiana, 70174

Skin and internal organ cells grow on fibers for burn victims and surgery repair. Cells on fibers are covered with patents, also when the fibers are rotated for fast reactions. There is no rejection when a patient gets his own cells, and cosmetics can be checked without killing animals. The National Cancer Institute has designated Taxol as an emergency priority because trees grow so slowly. By putting plant cells on rotating fibers, the cells grow faster. Some plant cells need light, and when a rotary biological contactor is run half full, the light hits a thin moving film. Patent 4,351,905 teaches cells on fibers and 4,407,954 teaches rotation. A Canadian patent will be issued soon describing entrapping Celite in the fibers for more area so more cells are immobilized. Tyvek fiber also has high area and is not expensive.

V-1092 Lead Ammonium Citrate Acetate (LACA) Prevents Spontaneous Apoptosis of Primary Adult Rat Hepatocytes in Synthetic Medium. M. Ribecco, E. Tognana, L.Testolin, L.Menapace, & U. ARMATO, Verona School of Medicine, I-37134, Italy.

Lead is a known environmental toxicant and a suspected carcinogen, whose cellular mechanisms of action are as yet poorly understood. Since *in vivo* lead nitrate given i.p. also acts as a hepatomitogen, we tested its possible direct proliferogenic effects by administering several doses (from 10^{-12} to 10^{-6} *M*) of the hydrosoluble complex LACA to primary adult rat hepatocytes kept in the $HiWo_5Ba2000$ synthetic medium. Here we report that LACA acted as a synchronizing agent rather than a true mitogen, shifting the peak of the spontaneously cycling adult hepatocytes from day 4 (controls) to day 5 *in vitro*. Instead, over a 10-day time lag LACA did reduce the spontaneous apoptosis otherwise occurring in the primary hepatocytes kept in the synthetic medium. Although the anti-apoptotic effect of LACA tended somehow to wane with time, after 10 days LACA-treated cultures contained, according to the dose used, from 30 to 60% more hepatocytes ($p < 0.02$) than did parallel controls. These results suggest that *(i)* the *in vivo* hepatomitogenic effects of lead are more likely to be indirect rather than direct; and *(ii)* the *in vitro* use of LACA may help to uncover biochemical mechanisms capable of preventing the occurrence of apoptosis in hepatocytes.

V-1093 Species Specificity of in vitro Transformation of Tracheal Cells by N-methyl-N'-nitro-N-nitrosoguanidine. M.J.W. CHANG and C.C. Tsai, Toxicology/ Pharmacology Lab, Chang Gung Medical College, Tao-Yuan, Taiwan, ROC

The literature has stated that hamster trachea is more sensitive to chemical carcinogenesis than rat. Primary cell cultures derived from tracheas of young Syrian hamsters and Wistar rats were characterized for proliferation rate, DNA repair synthesis, glutathione (GSH) content, and in vitro transformation. It was found that hamster tracheal cells grew slightly faster with a shorter doubling time and a higher CFE and had less GSH but more DNA repair synthesis induced by MNNG than rat tracheal cells. Only hamster tracheal cells were successfully transformed by MNNG. The transformed cells were able to grow in soft agar and developed into a malignant fibrous histiocytoma in the cheek pouch of a syngenetic animal. Higher DNA repair synthesis was interpreted as indicating that more DNA damage was induced since less intracellular GSH was available to detoxify MNNG. A tentative conclusion was drawned that the combination of more DNA damage and faster cell proliferation was likely the major attributor to the successful transformation of hamster tracheal cells. (Supported by CMRP 279, Chang Gung Medical College and NSC 80-0412-B-182-63, Taiwan, ROC.)

V-1094 A Microtiter Enzyme Linked Immunosorbent Assay For Protein Tyrosine Phosphatase. S. MISHRA and A. W. Hamburger*. University of Maryland Cancer Center, Division of Cell and Molecular Biology, 655 W. Baltimore Street, Baltimore, MD 21201

Protein tyrosine phosphatases (PTPases) have been determined to possess tumor suppressor function. We report the development of an enzyme linked immunosorbent assay (ELISA) for PTPases. Cellular PTPase activity was monitored by quantitating the disappearance of O-phospho-L-tyrosine (P-Tyr) in an ELISA system using antigen capture followed by double antibody labelling. The PTPase kinetics in crude cell lysates and in membrane preparations of MDA-MB 468 breast carcinoma cells were compared with those obtained by a standard $^{32}P_i$ release assay using radio-labelled Raytide™ as PTPase substrate. One milli unit of PTPase activity was defined as that amount of enzyme producing a rate of loss of 0.025 absorbance units/minute based on the unit of PTPase activity from the conventional assay system. The decrease in P-Tyr concentration was dependent on the time of incubation with the enzyme and on enzyme concentration and compared well with the release of $^{32}P_i$ in the radioactive assay system. Orthovanadate as well as heat denaturation of the enzyme inhibited the PTPase activity of the cell lysates significantly to similar degrees in both the assay systems. The assay presented here is a simple immunological system capable of measuring PTPase levels in cell and tissue extracts.

V-1095 CORRELATION BETWEEN INVITRO AND INVIVO INDUCED CARCINOGENICITY BY DDT. P.Balakrishnamurthy and K.Revathi Department of Toxicology, Fredrick Institute of plant protection and Toxicology,Padappai-601301 and Department of Zoology,Ethiraj college,Madras,India.

We studied the correlation between InVitro Cell transformation and InVivo Tumor Development by DDT. BALB/C mice were given High tollerable dose of DDT in Long Term Bioassay. Periodically peripheral blood samples were collected from mice and high molecular weight DNA was collected from the Lymphocytes. The DNA thus obtained was used to transfect NIH 3T3 cells in InVitro transfection assay. DNA from mice given DDT at the end of 3 months of feeding presented High degree of efficiency in transforming NIH 3T3 cells by exhibiting Foci,as compared to DNA from mice fed solvent alone. Those mice whose DNA showed Higher positivity for InVitro transformation also showed InVivo tumor development at the end of 10-12 months of feeding with DDT. In addition,Foci developed due to DNA Mediated transfection obtained from DDT fed mice,presented increased sister chromatid exchange levels.These Data thus indicate the utility of Transfection assay in predicting InVivo Tumor Development at much earlier time point of rodent Bioassay.

V-1096 **Screening of Chemopreventive Agents Using the A427 Human Tumor Cell Anchorage-Independence Assay.** E.A. Korytynski, S. Sheela, and V.E. Steele*. ManTech Environmental Technology, Inc., Research Triangle Park, NC 27709 and *Division of the National Cancer Institute, Bethesda, MD 20892.

An anchorage-independence assay (AIA) with human lung tumor cells, A427, was used to screen potential chemopreventive agents. The cells were seeded in soft agarose with and without test agent and maintained for 28 days. 13-*cis*-Retinoic acid was used as the positive control for the 140 agents tested. A cytotoxicity assay was performed concurrently and toxic doses were not considered in the determination of an agent's response. Of the potential chemopreventive agents tested in the A427 AIA, 19 or 23 anti-inflammatory agents, 11 of 18 cyclo-oxygenase inhibitors, 4 of 5 cAMP enhancers/phosphodiesterase inhibitors, 14 or 22 ODC inhibitors, 8 of 15 glutathione S-transferase inhibitors, 14 of 24 free radical scavengers were positive. Although many agents fell into more than one category, selecting only one activity was inappropriate. The A427 AIA was able to screen chemopreventive agents with different biological activities. This work was supported by NCI Contracts NO1-CN-55503-02, NO1-CN-55503-04, NO1-CN-95172-01, and NO1-CN-95172-05.

V-1097 ***In Vitro*** **Prescreening of Chemopreventive Agents using Assay for GSH and Free Radical Formation.** J.D. Stutzman, K. Garris, and S. Sheela. ManTech Environmental Technology, Inc., Research Triangle Park, NC 27709.

We have screened a large number (152-169) of compounds for potential chemopreventive activity using six biomarker assays. The results of two assays, induction of GSH and inhibition of free radical formation are presented here. Reduced glutathione (GSH) is a thiol containing tripeptide that plays an important role in the detoxification of some carcinogens, the protection of cells from free radical damage, and a variety of other cellular functions. It has been suggested that oxygen radicals are involved in the initiation and promotion of carcinogenic processes. Potential chemopreventives were evaluated for their ability to enhance GSH production by exposing confluent Buffalo Rat Liver (BRL) cells to various concentrations of a drug for 24 hours. Following incubation the GSH levels in cell free extract were determined by reacting it with O-phthalaldehyde (OPT) to form a highly fluorescent product that is activated at 350nm with an emission peak at 420nm and is read on a fluorometer. By comparing the level of fluorescence in chemopreventive agent (CPA) treated samples to that in untreated samples, the GSH levels were determined. Free radical formation was induced in primary human fibroblast or leukemic cells (HL-60) by addition of 12-O-tetradecanoyl phorbol-13-acetate (TPA) alone or TPA with five doses of a CPA and incubated at 37°C for 20 min. The level of free radical formation was measured by the reduction of cytochrome C. Using TPA alone as a measure of maximum free radical formation, the percent of maximum radical formation in CPA treated cells was calculated. The data of five CPAs tested in these two assays is presented. The results of antineoplastin A10, apigenin, and progesterone show an increase in GSH levels and a decrease in free radical formation, indicating that the reduction of free radicals may be due to the increased levels of GSH. However, the results of Sodium butyrate clearly show a reduction in free radical formation with no increase in GSH levels. This illustrates that there are obviously other mechanisms by which free radical formation can be reduced. It is also illustrated that GSH levels can be enhanced without necessarily impacting the level of free radicals formed as is the results with N-acetyl cysteine. These examples show that these assays used individually or in conjunction can be good screening assays for potential chemopreventives, but a more thorough investigation would be required before drawing any conclusions about the mechanisms involved. This work was supported by NCI Contract N01-CN-95172-03.

V-1098 **Biological Activity and Chemical Class of 125 Chemopreventive Agents in Relation to Efficacy in the Rat Tracheal Epithelial Cell Foci Inhibition Assay.** B.P. Wilkinson, J.T. Arnold, S. Sheela, and V.E. Steele*, ManTech Environmental Technology, Inc., Research Triangle Park, NC 27709, and *Division of the National Cancer Institute, Bethesda, MD 20892.

One hundred and twenty-five natural and synthetic agents that have potential cancer chemopreventive activity were evaluated using a rat tracheal epithelial (RTE) primary cell assay. The assay measures inhibition of benzo[a]pyrene (B[a]P)-induced morphological transformation in RTE cells. Freshly isolated RTE cells were exposed to B[a]P alone or in combination with the test chemopreventive agent. After 30 days in culture, transformed foci were scored and inhibition was quantitated. In these studies, foci formation was inhibited by agents which have been described to modulate the initiation stage of carcinogenesis. It was especially sensitive to agents that enhance glutathione levels (80% agents with this activity were positive) and glutathione-s-transferase activity (64% were positive), both of which are involved in carcinogen-detoxifying mechanisms. Among chemical classes, thiols tend to be positive in this assay (80%), as well as flavonoids (66%) and retinoids (72%). Out of 125 agents, 70 were classified as anit-initiators and 71% of these were positive, and of 39 agents considered anti-promoters, 33% were positive. The RTE assay had a 72% correlation with a hamster lung tumor model, correctly identifying 18/25 agents tested and is thus a good predictor for in vivo testing of potential chemopreventive agents. This work was supported by NCI Contracts N01-CN-55503-03, N01-CN-55503-05, N01-CN-95172-02, and N01-CN-95172-06.

V-1099 **Evaluation of Potential Chemopreventive Agents using Inhibition of Carcinogen-induced Calcium Tolerance Assay.** G.P. Wyatt, E.L. Elmore, S. Sheela, and V.E. Steele*. ManTech Environmental Technology, Inc., Research Triangle Park, NC 27709 and *Division of the National Cancer Institute, Bethesda, MD 20892.

The study of epithelial cells and their growth mechanisms are of particular interest because most adult human cancers are carcinomas. This assay was developed as an *in vitro* screening system for potential chemopreventive (CP) agents selected by NCI's Division of Cancer Prevention and Control using primary normal human foreskin epithelial cells. These cells were repeatedly exposed to a direct carcinogen, propane sultone (PS), alone and in combination with a chemopreventive agent (CPA) over several subcultures. The cells were then plated into various concentrations (8.8, 17.5, and 35μg/mL) of calcium and assessed for increased calcium tolerance, a marker for the carcinogen-induced change, by observing cell growth. Controls, PS alone and a positive control, PS with the addition of all-trans retinoic acid, were included with each experiment. A preliminary range-finding study was done for each CPA. Twelve compounds with varying biological activity were evaluated in this system resulting in all twelve showing inhibition of PS-induced calcium tolerance by 20% or more. This work was supported by NCI Contract N01-CN-95172-04.

V-1100 The Adaptation of Rabies Virus in Vero Cell and in Human Diploid Cell Lines. Wu TAI-CAI. Department of Virology, Anti-epidemic Center of Guangxi. Nanning, 530021, P.R.China.

The "aG" rabies virus strain, a Chinese rabies fixed virus used for prepared primary hamster kidney (HK) cell was continuously passaged in Vero cell 15~30 passages and 20~35 passages in human diploid cell lines. Some adapted strains were cloned by the terminal dilution method and passaged again through mouse brain for enhancing the virus titre. One adapted strain grew in vero cell and another adapted strain grew in human diploid cell lines and the virus titres in the supernatant increased step by step from 10^3/ml and 10^4/ml to 10^5/ml~10^7/ml (LD) separately. The incubation period of these strains in the two cell lines has been shortened from 10~14 to 4~7 days. The antigenicity of these virus strains did not show significant variation. The potency of these adapted strains was higher than HK cell line vaccine. The adapted strains also showed good ability to protect the mice against challenge by the fixed "aG" virus strain. The ED50 value was 1:120~1:160 in adapted strains and 1:50 in the HK vaccine. The results suggested these adapted strains might be promising as candidates for vero cell and human diploid cell rabies vaccine production.

V-1101 A murine myelomonocytic leukemia cell line resistant to ciprofloxacin. A.PESSINA,E.Mineo,L.Gribaldo,and M.G. Neri. Institute Medical Microbiology, University of Milan, Italy.

A subline of a murine myelomonocytic leukemia (WEHI-3B) resistant to ciprofloxacin (CPX) was selected to study the mechanisms by which the quinolones affect the mammalian cell functions. The IC50 of CPX for the resistant clone was 17.3 times higher than the IC50 for the parental strain (34 mg/l). No significant differences were evidenced when the two lines were compared for their proliferating capacity (by a multiwell MTT assay), agar clonogenicity, and CSFs production. The variant line expresses CPX resistance for 12 months, in the absence of the selecting agent, suggesting genetic stability. Surprisingly, the CPX resistant variant did not show any cross resistance to the other quinolones tested (ofloxacin, rufloxacin, pefloxacin, lomefloxacin). We are studying the intracellular uptake of quinolones by the CPX-resistant variant and by the parental strain in order to verify if the resistance may be related with the expression of some specific membrane protein (as P-glycoprotein in multiple drug-resistance) or to a decreased susceptibility of cell topoisomerases II to CPX.

V-1102 Growth characteristic and cytogenetic analysis of cellular clones derived from LoVo cells with intrinsic different sensitivity to doxorubicine. T.DASDIA*, M.ROMAGNONI*, E.SCANZIANI**, C.BARBIERI and E.DOLFINI*. *Ist.Mario Negri,v.Eritrea 62, Milano; **Ist.Anat.Patol. Veter., University Milano, Italy.

Doxorubicin (Dx) resistant cell lines are usually obtained from a parental line (WT cells) after long term exposure "in vitro" to the drug. Drug related effects which could play a role in the development of the resistant phenotype include: the mutagenic potential of Dx; the expression of some properties shared by the whole cell population; the selection of a cellular subline intrinsically resistant to Dx. To verify the last hypothesis we selected three cell clones from a human colon adenocarcinoma cell line (LoVo WT). The parental cells and the clones C13,C15, and C17 were characterized by sensitivity to Dx, growth kinetics, cytokeratin expression, and cytogenetic analysis. Results demonstrate that cells with different sensitivity to Dx (RI from 0.4 to 3) intrinsically exist in LoVo WT cell population. Differences in numerical and structural chromosome alterations are evident. Monosomy 2 and trisomy 7 are common to all cell lines. Monosomy 14 and 15 with translocation t (14q;15q) is present only in C13, the most sensitive do Dx. Cytokeratin filaments are immunohistochemically demonstrable in all cell lines.

V-1103 The Cell Line Data Base gives detailed information on human and animal cell lines. B. PARODI, A. Manniello, O. Aresu, P. Romano°, B. Iannotta, G. Rondanina and T. Ruzzon. Istituto Nazionale per la Ricerca sul Cancro, 16132 Genova, Italy and °Istituto di Oncologia Clinica e Sperimentale, Università di Genova, 16132 Genova, Italy

Within the Interlab Project, aimed at the creation of databases of biomedical interest, the Cell Line Data Base (CLDB) was set up. It is available on-line, and contains detailed information on human and animal cell lines obtainable by European cell line collections. Data are referred to about 2000 distinct cell lines, and include identification (name, growing conditions, culture type, typology, tumorigenicity, clonality, karyology, mutations, morphology, bibliography); origin (species, strain, sex, tissue, tumor, pathology, original line, transformation); specification (applications, specific functions, endogenous viruses, virus susceptibility); ownership/culture (laboratory, passage, mycoplasma assays, validation assays, advices, culture conditions, freezing medium) and availability (catalogue, catalogue code). Besides the most widely used cell lines, two major groups of lines are described: human and animal cell lines derived from tumors, and human cells relative to a wide range of genetic diseases (about 150 different diseases, as defined by McKusick). A large majority (more than 80%) of the cell lines are not described in the most widely known catalogues. The data format of CLDB has been adopted by the Working Committe for a European Database of Animal Cell Lines, and a personal computer version of the database was prepared, in order to make data collection easier, and to help in the management of local cell line collections. The first edition of a written catalogue has also been produced, and the second edition will be available by december 1992.

V-1104 Gertrude C. Buehring
University of California, USA

Effects of Bovine Leukemia Virus on the Growth and Differentiation of Bovine Mammary Epithelial Cells

READ BY TITLE

V-1105 ANIMAL CELL CULTURE COLLECTION IN RUSSIA
I. I. FREEDLASKAVA, G. G. Polanskaya, T. N. Efremava and G. P. Pinaev. Cell Culture Department, Institute of Cytology, Russian Ac. Sci. Tikchoretsky av.4, S.-Petersburg 194064, Russia

The aim of the Collection is gathering, preservation, characterization and distribution of animal and human permanent cell lines. Cell line characteristics are close to those estimated in other animal cell culture collections. Growth parameters, functional traits, tumorigenecity, mycoplasma contamination, karyotype, species- and intra-species identity are determined. The number of items in the Collection now includes about 700 cell lines and hybridomas. Cells are preserved in liquid nitrogen under strictly controlled conditions. Effect of cryoconservation on genetical stability of cell lines has been investigated. Researches to improve methods of cell quality control are carried out. They concern genetical stability during long-term cultivation of animal cell lines, mycoplasma detection and eradication, intra-species cell line variability. Information on cell lines and hybridomas is available through Data Bank.

V-1106 Fish Cell Culture as a Mean in Studying Fish Reproduction. J. Galas P.Epler, S. STOKLOSOWA, Laboratory of Animal Endocrinology & Tissue Culture, the Jagiellonian University, 30-060 Krakow Poland.

Follicular (FC) and interstitial (IC) cells were isolated from carp ovary in April and December. Trypsin was used to obtain cell suspension. The cells were then separately cultured as monolayers for 6 days. Culture media were changed every second day. Progesterone (P_4), estradiol (E_2) and androgen (A) secretion into the culture media was estimated by appropiate radioimmunoassays. There were significant differences in the secretion of the 3 steroids between (FC) and (IC). There was also a significant difference in the hormonal activity of both investigated cell types between the cells isolated in spring and in winter. SPRING - P_4-FC-125 pg/culture and IC-45 pg respectively, E_2-FC-1307 pg and IC-305 pg, A-FC-no secretion and IC-35 pg. WINTER - P_4-FC-60 pg/culture and IC-100 pg, E_2-FC-476 pg and no secretion, A - FC-no secretion and IC-15 pg. In winter, IC were more active in respect to A secretion while in spring, FC produced more P_4 and E_2. The cells responded to gonadotropic stimulation. The results obtained showed that cultured carp ovarian cells are a suitable tool for the research of fish reproduction.

V-1107 Do Sertoli Cells Cocultured with Leydig Cells Influence the Arrangement of their Cytoskeleton? B. BILIŃSKA. Laboratory of Animal Endocrinology & Tissue Culture, the Jagiellonian University, 30-060 Krakow, Poland.

Since it is known that Sertoli cells modulate steroid production by Leydig cells in vitro, an effort was undertaken to see whether the cytoskeleton is involved in this process. Co-cultures of Leydig and Sertoli cells were examined in light microscope (Nomarski optics) and in scanning electron microscope. To discriminate each of the two cell types, histochemical test for $\Delta^5,3\beta$ -HSD activity was performed. To visualize the cytosketal elements immunofluorescence staining was carried out using monoclonal antibodies. The microfilaments were stained with rhodamine-phalloidin. In co-culture three main effects were observed: 1) rise of androgen secreted by Leydig cells, 2) changes of Leydig cell-shape, 3) increase of enzyme activity. Concurrently, changes in the microfilament organization were seen. The stress fibers visible in Leydig cells cultured alone, were dispersed or disappeared in co-culture. This effect was strenghtened in the presence of FSH in culture medium which was not the case in monoculture of Leydig cells. The results were discussed in respect to a possible stimulation of Leydig cell function by Sertoli cells in vitro.

V-1108 Histamine-induced Contrasuppression in Bone Marrow. N.BELYAEV, G.Zakiryanova,A.Beklemishev. Inst.of Molecular Biology & Biochemistry, 86 Michurin st. ALMA-ATA 480012 USSR

Production of suppressing factors was studied under pretreatment of mice bone marrow cells (BMC) with 10^{-5}M histamine (H) for 3 hours. H substantially deteriorated suppressing effect of 48 hr BMC culture medium on X63.Ag8.653 myeloma cells (by incorporation of ^{14}C-timidine into DNA). The effect distinctly observed in BMC of WR/y, was visible in BALB/c and invisible in B10 cw/y mice lines. BMC were subdivided into 6 fractions (F) by percoll gradient separation. Except F3 (ρ=1,076), all F showed suppressing effect. F3 controled the synthesis of mitogenic factors in BMC. H-binding cells were found among F1 (1,033), F3, F4 (1,090) and F6 (>1,100) by ^{125}I-labeling with BSA-conjugated H. However, only F1 and F4 supernatants induced H-mediated contrasuppression. This stage was provided by a special soluble factor, but not H. H inhibited BMC proliferation in F1, F4 and increased it in F3 and F6. F6 facilitated secretion of the suppressing factor after H induction. H is suggested to be an intermediate in complex regulation of BMC proliferation. Bone marrow mast cells, induced with IL-3, may play the role of endogenous H source.