SYMPOSIUM

S-1. The Decapentaplegic Gene Complex in Drosophila. W. M. GELBART, F. M. Hoffmann, V. Irish, L. Posakony, D. Segal, F. Spencer and D. St. Johnston, Dept. of Cellular and Developmental Biology. Harvard University, Cambridge, MA 02138

The decapentaplegic gene complex encodes a number of functionally integrated activities necessary for normal development in Drosophila. Extensive genetic analysis has allowed us to delineate three major regions of the complex; shv (shortvein), EL (embryonic lethal) and dpp (decapentaplegic). Based on the phenotypes elicited by the mutations in these various regions, we are presently entertaining the hypothesis that this gene complex controls the elaboration of positional information in epidermal structures and perhaps other tissue systems in Drosophila. Using a mobile genetic element inserted near decapentaplegic, we were able to begin a "chromosomal walk" through the decapentaplegic complex. Recombinant phage containing sequences from throughout the complex have been identified. As most decapentaplegic mutations are gross chromosomal rearrangements, the physical mapping of mutations has been straightforward. At present, the decapentaplegic gene complex appears to reside within roughly 50 kilobases of DNA.

(Supported by NIH Grant GM 28669.)

S-2. The Molecular Basis of a Simple Behavior. Richard H. SCHELLER, Department of Biological Sciences, Stanford University, Stanford, CA 94305

Egg laying in the gastropod mollusc Aplysia is an example of a classic neuroendocrine fixed action pattern. The behavior is characterized by a cessation of locomotion, an inhibition of feeding and increased respiratory pumping followed by head waving and egg deposition. This multicomponent action pattern is thought to be generated by a central nervous system program which is activated by a battery of neuropeptide transmitters. We have isolated and characterized a family of genes which encode these peptides. Different members of the gene family are specifically expressed in the atrial gland, an exocrine tissue, and the central nervous system. Each gene encodes a polyprotein in which multiple neuroactive peptides are released from the primary translation product by internal proteolytic cleavages at basic residues. The differences between genes are clustered in regions that determine the pathway of post-translational cleavage and modification. As a result even though the genes are about 90% homologous each expresses a unique set of related peptides. The behavior is generated as the peptides alter the firing patterns of cells in the central nervous system. At the same time a physiological response occurs via hormonal actions as the molecules diffuse into the hemolymph and are dispersed through the organism. From these studies we conclude that the polyprotein is an evolutionary unit which coordinates a specific behavior with a physiological activity via the simultaneous synthesis and eventual release of multiple neuroactive peptides and hormones.

S-3. Activation of the myc Oncogene by Chromosomal Translocation. M. D. COLE, G. L. C. Shen-Ong, E. J. Keath and S. P. Piccoli, St. Louis University School of Medicine, 1402 S. Grand Blvd., St. Louis, MO 63104

DNAs from normal eucaryotic cells contain genes which are the progenitors of the transforming sequences in the acute oncogenic RNA tumor viruses. We have found DNA rearrangements associated with the c-myc oncogene in eleven out of thirteen mouse plasmacytomas examined. Molecular analysis of clones of the plasmacytoma myc genes showed that the rearrangement joined myc to the immunoglobulin a heavy chain constant region gene (C\(_{\alpha}\)), probably through an abortive "switching" event. Since C\(_{\alpha}\) is on chromosome 12 and c-myc has recently been localized to chromosome 15, this oncogene rearrangement corresponds to the 12:15 chromosomal translocation characteristic of mouse plasmacytomas. Translocation was found to correlate with the production of a tumor-specific RNA that is 0.4 kb shorter than the normal mRNA. Electron microscopy of interspecies DNA hybrids has identified two potential myc exons that are displaced by the translocation; thus the translocation has altered the myc oncogene product which may be related to tumor formation. Furthermore, no elevated levels of myc RNA were found in plasmacytomas when compared to normal B lymphocytes. Studies in many tumor systems suggest that chromosomal translocation may be a common mechanism for activation of cellular oncogenes in the induction of neoplasia.

S-4. Mechanisms of Formation of Human Oncogenes. H. Land, L. F. Parada and R. A. WEINBERG, Center for Cancer Research and Department of Biology, Cambridge, MA 02139 and The Whitehead Institute for Biomedical Research, Cambridge, MA 02139

Gene transfer experiments have allowed definition of a series of oncogenes that are present in human tumors. These oncogenes are detected by their ability to transform the NIH3T3 mouse fibroblasts. Each of these genes arises from alteration of a normal cellular gene termed a proto-oncogene. The oncogenes are able to morphologically transform primary embryo fibroblasts, but resulting colonies are poorly tumorigenic, unless adapted subsequently by extensive in vitro passaging. The data indicate that during establishment in vitro cells acquire functions that complement the oncogene and thus enable the full tumor cell phenotype to be displayed. These complementing functions may be provided by other cloned oncogenes of viral origin. Analysis of cloned cellular and viral oncogenes indicates the existence of several functional classes of genes each of which has distinct effects on cellular phenotype.

WORKSHOPS

W-1 through W-8. We do not publish abstracts of these presentations.

ROUND TABLES

R-1 through R-11. We do not publish abstracts of these presentations.
1. Dreams of Cellular Physiology Captured in Tissue Culture Systems — George Gey’s Bequest. R. A. PATTILLO and A. C. F. Ruckert, The Medical College of Wisconsin, 8700 West Wisconsin Avenue, Milwaukee, WI 53226

George Gey not only dreamed, but translated those dreams over the course of more than half a century into the fundamental cornerstones of tissue physiology in living systems in vitro. This discourse will follow a scientist’s perceptions of how living tissue culture systems glimpse cellular physiology through the fleeting frames of dynamic events in life processes. The paper focuses upon the phases of Gey’s scientific career beginning with the move from Johns Hopkins University to Milwaukee, Wisconsin. From 1929 to 1951, of his methodologies translated into the establishment of the HeLa cell system and its being made available for propagation of the polio virus. This led to the resolution of one of health’s major tragedies, poliomyelitis. Establishment of the roller tube method would form the basis for development of a unique tissue culture system where explant cultures could take their place as a monumental contribution to cell science. The next phase in Dr. Gey’s scientific life was the securing of a permanent home for the Tissue Culture Association. This provided a cell science center for scientists around the world with expertise in a discipline to be utilized in addressing problems of human physiology and its aberrations. The W. Alton Jones Foundation was the answer to this dream. The Tissue Culture Association and its outstanding membership continues to fulfill the dreams of many like George Gey, who believed that the truth of science could be witnessed and captured in living tissue culture systems.

2. Human Cancer Cell Culture. G. E. MOORE,* Department of Health and Hospitals, Denver, CO 80204

My cell laboratory was started because of a personal challenge. A cynic commented that our isolation of tumor cells from the blood was meaningless until we could prove that they were alive. The resultant cell culture studies evolved and changed as a result of subsequent specific scientific challenges. Media have been designed for human lymphoid and epithelial cells. The RPMI and GEM series now numbers 1749; no originality is claimed. For example, the RPMI #1640 medium was “designed” to grow normal human lymphocytes at a time when many scientists thought that only malignant cells could be established as long term cell lines. The premature design of a cell plant for the culture and daily harvest of 2-4 kilograms of human lymphocytes illustrates some of the continuing technical and fundamental problems of cell culture. About 75 kilograms of cells were harvested in one year. I will review attempts to institute cell therapy for malignant disease and genetic disorders and the limitations of current research involving interferons, cell products, and monoclonal antibodies. A perspective of cell culture studies for the years 1966-2000 was published in 1967. It has not been fulfilled but it is interesting from a retrospective viewpoint. A new perspective of cell culture will be provided.

3. Establishment of Cell Lines from Human Solid Tumors. A. LEIBOVITZ*, University of Arizona Cancer Center, Tucson, AZ 85724

Tumors in advanced countries by means of sanitation, vaccination and antibiotics. No progress had been made against a group of virus infections including hepatitis, measles, herpes zoster, varicella, common cold, polio-myelitis, mumps and a battery of entero, roto, and ECHO viruses yet to be identified. All were strict parasites of man and could not be propagated in the laboratory or in any common experimental animal. This report will review the rationale for use of human cell culture as a vehicle for propagation of some of these viruses, use of antibiotics for direct virus isolation, and some of the problems and solutions associated with HeLa cells; microbial contamination; transformation of long term normal cell lines; species contamination; PPO contamination; the mechanisms of cell culture contamination and procedures for prevention including aseptic techniques, antibiotics, laminar flow filtered air techniques, and storage of cell cultures in liquid nitrogen.

4. Problem Solving with Cell Cultures. L. CORIELL*, Institute for Medical Research, Copewood Street, Camden, NJ 08103

Recent advances in the composition of culture medium have greatly enhanced the possibility of establishing continuous cell lines from human solid tumors. However, inherent difficulties exist in the establishment of tumor lines, including: 1) tumor cell heterogeneity, 2) admixtures of normal reactive cells and stromal elements, 3) inadequate tumor cell viability, and 4) appropriate media and substrate requirements for sustained in vitro growth. In order to try to optimize the possibility of monolayer growth, we have developed a transport media, and basic culture media which appear suited for growth of a variety of human tumors. Principle constituents of both the basic and transport media include insulin, transferrin, hydrocortisone, selenium, polyvinylpyrrolidone (PVP-360) and 2% fetal bovine serum. Additionally, we have recently developed a non-cellular destructive tumor disaggregation technique which utilizes a hypotonic medium. Also, we recently have studied >100 solid tumors for their ability to grow on plastic in monolayer culture, and compared this to their ability to form colonies in soft agar. Identical success rates for initial tumor growth in both agar and plastic were observed (p<0.01). Finally, our recent studies have suggested that continuous cell lines can be established from tumor cells initially cloned in agar suggesting that agar-prescreening may be useful in isolating tumor cells with high proliferative potential.

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5. Remembrances of Things Past Or The Coming of Age of WI-38. L. HAYFLICK,* Center for Gerontological Studies, University of Florida, 3357 GPA, Gainesville, FL 32611

The early history of human diploid cell strains is a litany of failures and disappointments. The original strains,
developed more by serendipity than by design, were lost in a freezer failure a few months before the paper describing them appeared. Our discovery of their finite replicative capacity (Phase III) encountered a dogma that was entrenched for 50 years. The paper describing them was rejected because "the largest fact to have come out from tissue culture in the last 50 years is that cells inherently capable of multiplying will do so indefinitely..." Our suggestion that Phase III might be related to aging was dismissed as "notably rash." Our claim that the cells could be useful in virology and as substrates for vaccines was regarded as "extraneous." Despite this unauspicious beginning, the Phase III phenomenon has been confirmed in laboratories worldwide and has given rise to the field of cytogerontology with new insights into the biology of aging. WI-38 has now come of age; it is 21 years old this month. Over one million cultures have been used by virologists throughout the world. WI-38 was the first non-primary and human cell population to be used as substrate for human virus vaccines. These have benefited tens of millions of people against such diseases as poliomyelitis, rubella, adenoviruses, CMV and rabies. Experiences such as these are not unique in science but young investigators who may face discouragement must be reminded of them periodically.

6. Intermediate Filaments and Cell Diversification. H. HOLTZER, G. Bennet, P. Antin, S. Tapscott and M. Pacifici, Department of Anatomy, University of Pennsylvania, Philadelphia, PA 19104

Much is known of the different intermediate filaments on a molecular level; nothing is known of their function(s). Here we focus on the "switches" of synthesis and intracellular localization as cells pass from compartment to compartment in the erythropoietic, neurogenic and myogenic lineages. Virtually all cells in the 12-15 hour chick blastodisc are cytokeratin-positive; they are vimentin-, desmin- and neurofilament (NIF)-negative. When dispersed and reared in culture for 12-18 hours, roughly 5-10% of the population become cytokeratin-negative and vimentin- and hemoglobin b-positive. All cells that exhibit this switch undergo one round of DNA synthesis. All cells (100%) in the neural tube of a 48 hour chick embryo are cytokeratin- and NIF-negative but vimentin-positive. During their terminal cell cycle presumptive neuroblasts initiate the synthesis of NIF. Both their postmitotic daughters lose their vimentin, remaining NIF-positive. The 160K subunit of NIF appears and rapidly disappears in early compartments of the retinal pigment and lens lineages. All (100%) presumptive myoblasts in limb buds, somites, and 10-day muscles are exclusively vimentin-positive. During their terminal cell cycle they initiate the synthesis of desmin. Their postmitotic daughters and/or myotubes display longitudinally-oriented vimentin and desmin filaments. With maturity the longitudinal vimentin filaments disappear and the desmin localizes transversely to the I-Z band region.

7. Neural Cytoskeleton. M. L. SHELANISKI, New York University School of Medicine, New York, NY 10016

The cytoskeletons of neural cells, in common with other somatic cells, are composed of microtubules, intermediate filaments, microfilaments and a variety of cross linking bridges. They differ from these cells in that the intermediate filaments (IF) of two of the major neural cell types—neurofilaments in neurons and glial filaments in astroglial cells—differs from both the cytokeratins and vimentin and are specific for these cell types. The neurofilament (NF) is composed of a core protein with a molecular weight of 68,000 and two tightly associated peripheral proteins of 150K and 200K molecular weight. The astroglial filament (GF) is composed of a single protein with MW=51,000. Embryonic neurons and glia both show the presence of vimentin in IF which are eventually replaced, in part or in toto, by NF or GF. Thus, these three intermediate filament types can serve as useful markers of differentiation and of cell type. The highly asymmetrical architecture of the neuron places significant morphological constraints on its cytoskeleton and great demands on it for the transport of synthesis products from the cell body to the distant tips of axons and dendrites. The mechanisms by which the cytoskeleton meets these needs and the data on interactions between individual elements of the cytoskeleton will be presented. Specifically, the possibility that microtubule-associated proteins (MAPs) mediate interactions will be discussed. The possible role of these interactions in the pathogenesis of disease will be examined.

8. Keratin Classes as Molecular Markers for Different Types of Epithelial Differentiation. T.-T. SUN,* Departments of Dermatology and Pharmacology, New York University School of Medicine, New York, NY 10016

Keratins are a group of water-insoluble proteins (MW 40-70K) which form intermediate filaments in a wide variety of epithelial cells. The subunit composition of the keratin filaments varies with cell type, embryonic development, histologic differentiation, cellular growth, and disease state. To better understand the functional significance of individual keratin species, we have used several monoclonal anti-keratin antibodies to localize specific keratins in normal human epidermis and to survey the keratins expressed by various in vivo epithelia. Immunolocalization results indicate that in skin epidermis a 50K and a 58K keratin are present in all living layers, whereas a 56.5K and a 65-67K keratin are associated only with the more differentiated cells above the basal layer. Tissue-distribution data further showed that the numerous keratin species identified so far can be grouped into a relatively small number of classes according to their immunological reactivity and size. Among the keratin classes, the 50K and 58K classes appear to be characteristic of all stratified squamous epithelia, whereas the 56.5K and 65-67K classes are unique to the keratinized epidermis. These findings suggest that specific keratin classes as defined by monoclonal antibodies may serve as markers for different types of epithelial differentiation (simple vs. stratified; keratinized vs. non-keratinized). The application of such a concept for determining the in vivo origin of various cultured epithelia will be discussed.
9. Intermediate Filaments as Markers for Determining the Origin of Cell Lines and of Human Tumors. MARY OSBORN, Elke Debus, Michael Altmannberger and Klaus Weber, Max Planck Institute for Biophysical Chemistry, and Department of Pathology, University of Gottingen, Gottingen, FRG

Intermediate filaments (IFs) are a multigene family of proteins which share related but distinct sequences. Cells can be subdivided into six types by IF typing, i.e. (1) epithelial cells (cytokeratin +), (2) most neurons (neurofilament +), (3) muscle cells (desmin +), (4) glial cells (GFAP +), (5) cells of mesenchymal origin (vimentin +), (6) a few cell types which appear to lack IFs. IF typing is therefore a powerful tool to determine the origin of cell lines in culture, or to determine what cell type predominates in primary or secondary cultures. Prototype cell lines expressing each IF type are known. A wide variety of human tumors have been examined both by histological and by cytological methods. IF typing distinguishes the major tumor groups, i.e. (1) carcinoma (cytokeratin +), (2) sympathetic derived tumors such as ganglionneuroblastoma and pheochromocytoma (neurofilament +), (3) rhabdomyosarcoma (desmin +), (4) glioma (GFAP +), (5) non-muscle sarcoma (vimentin +). Further subdivision of epithelium and of carcinomas is possible using monoclonal antibodies, which distinguish between different cytokeratin polypeptides.

10. The “Mesothelial Keratins”: Expression and Regulation in Cultured Epithelial Cell Types and in vivo. J. G. RHEINWALD, Y.-J. Wu, N. D. Connell, O. Alberti, and P. J. LaRocca, Dana-Farber Cancer Institute and Department of Physiology and Biophysics, Harvard Medical School, Boston, MA 02115

Recent comparison of the Triton-insoluble protein fractions of cultured human epithelial cell types has revealed the existence of a new family of keratins (40, 44, 52, and 55 kDa). We have named these “mesothelial keratins” because they constitute the set of keratins synthesized by mesothelial cells, and are different in size, charge, and/or sequence from the keratins (46, 50, 52, 56, and 58 kDa) synthesized by epidermal keratinocytes. Our studies of epithelial cell types in culture and studies by others of in vivo epithelia have revealed that the mesothelium and epidermis are extremes; most other epithelia synthesize some mesothelial as well as some epidermal keratins, in tissue-specific patterns. A specific antiserum to the 40 kD keratin has disclosed its presence in many epithelia in vivo, with a distribution that provides clues to the origin of neoplastic cells in the cervix and ovary, and to the nature of cell heterogeneity in the mammalian epithelium. The in vivo keratin patterns resemble but are usually not identical to those expressed by the respective cell type in culture. It is clear now that certain subsets of keratin genes are opened for expression as a feature of the differentiated state, but that some of the keratins are expressed constitutively and some are subject to regulation in each epithelial cell type. In the mesothelial cell, for example, the synthesis of all four keratins is repressed during rapid growth but induced to high levels when cells are quiescent, in a reversible fashion.

11. Somatic embryogenesis in grasses. Indra K. VASIL, Department of Botany, University of Florida, Gainesville, FL 32611

There are three principal methods of plant regeneration in vitro: (a) development of axillary shoot meristems, (b) de novo formation of shoot meristems, and (c) somatic embryogenesis. The formation of somatic embryos has been found to be more attractive because of the rapidity with which large populations of genetically identical plants can be obtained within a short time. Unfortunately, the formation of somatic embryos has not been generally possible in many important crop plants. We have obtained extensive evidence of somatic embryogenesis in callus, cell suspension and protoplast cultures of a variety of grass species, which include sugarcane and cereal crops like wheat, maize, millets, etc. The plants obtained from the somatic embryos as remarkable uniform and genetically indistinguishable from the donor plants. No evidence of genetic variability induced during the culture period was found in plants obtained from somatic embryos. The significance of the single cell origin of the somatic embryos, the lack of genetic variability and the many possible uses of embryogenic cell cultures in genetic modification experiments will be described and discussed.

12. Asexual Embryogenesis in Palms. BRENT TISSERAT, U.S. Department of Agriculture, Agriculture Research Service, Western Region, Fruit and Vegetable Chemistry Laboratory, Pasadena, CA 91106 (Sponsored by I. K. Vasil)

The genetics, biochemistry, physiology, and morphogenesis of tree crops are neglected compared to herbaceous species. Tree crops such as the palms (Areaceae) are difficult to study due to their long-life, habit, habitat, and highly heterozygous genetic nature. In vitro techniques appear to be promising tools to study palm growth and development compared to field and greenhouse experimentation. Palm vegetative propagation methods are often insufficient to obtain immediate large clonal acreage plantings. Further, many palms do not exhibit any natural means of asexual propagation (e.g. oil and coconut palms). Potentially, tissue culture techniques may be used to parlay desired clones into large, uniform, clonal populations within a few years. Palm plantlets may be produced through either asexual embryogenesis, i.e. initiation and germination of somatic embryos from callus, or organogenesis, i.e. rooting and division of shoot tips and lateral buds. Asexual embryogenesis appears to offer the more plausible method of cloning most palms due to the high plantlet production potential from callus and absence of vegetative buds in many palms. A discussion of the asexual embryogenesis processes in palms is presented. Large tissue-culture derived tree populations could be useful to critically study the biochemical and physiological action of growth regulators in both sterile and field conditions compared to seedlings. Both suckering and flowering phenomena have occurred in vitro from palm plantlets. Elucidation of these growth processes could be used to stimulate desired growth responses on demand in the field.
13. The Use of Macromolecular Markers to Study Plant Embryo Development in vitro. M. L. CROUCH, Department of Biology, Indiana University, Bloomington, IN 47405

There are two distinct pathways of regeneration in plant tissue cultures: regeneration of apical meristems which then organize their own shoot or root system, and regeneration of bipolar embryos. The two pathways can be distinguished not only on the basis of morphology, but also by the presence of macromolecules characteristic of embryo development. The rate of synthesis of embryo-specific macromolecules can be used to assess the progress of embryogenic cultures, and to monitor the effects ofchanging the culture environment. I have used antibodies specific for the 12S storage protein of Brassica napus to study the regulation of embryogeny in both zygotic and non-zygotic embryos in culture. Immature zygotic embryos will not synthesize storage proteins at the in vivo rate when they are removed from the seed and placed in culture, unless abscisic acid or high osmoticum is added to the medium. This indicates that the seed environment controls the rate of storage protein synthesis (Crouch and Sussex, 1981, Planta 153: 64-74). However, the seed is not necessary for initial synthesis. Nonzygotic embryos induced from microspores on a culture medium containing a high sucrose level synthesize storage proteins earlier than their zygotic counterparts, but at a much lower rate (Crouch, 1983, Planta, in press). We are currently using storage protein cDNA probes to monitor gene expression during initiation of non-zygotic embryos as a way to study early events, and during zygotic embryo culture to study regulation of embryo maturation.

14. Carrot and Capsella: mRNA Synthesis and Accumulation during Somatic Embryogenesis and Zygotic Embryogenesis. V. RAGHAVAN, The Ohio State University, Columbus, OH 43210

A comparative study has been made of the timing of synthesis or accumulation of poly(A)-containing RNA (mRNA) during somatic embryogenesis in a carrot cell suspension and during zygotic embryogenesis in Capsella bursa-pastoris. Analysis by acrylamide gel electrophoresis and affinity chromatography has shown that during somatic embryogenesis in carrot cells (elicited by removal of auxin from the medium) there was a decrease in the rate of synthesis of rRNA concomitant with an increased synthesis of 12S-18S RNA. However, embryogenic cells synthesized poly(A)-containing RNA at a higher rate than the nonembryogenic cells, suggesting that removal of auxin from the medium controls the biochemical events of embryogenesis by the synthesis of mRNA. In situ hybridization with [3H]polyuridylic acid ([3H]-poly(U)) has shown that fertilization was accompanied by an increased accumulation of [3H]-poly(U) binding in the egg of C. bursa-pastoris. Although there were no appreciable differences in [3H]-poly(U) binding activity between the terminal embryonal cell and basal suspensor cell of a two-celled embryo, subsequent divisions of the suspensor cell were accompanied by a gradual decrease and complete disappearance of [3H]-poly(U) binding sites in the daughter cells formed, while the embryonal cells continued to bind the label. These results suggest that mRNA is subject to differential accumulation in the cells during zygotic embryogenesis.

15. Biological Activity of Carbon Particles Coated with Benz(a)Pyrene and Pyrene in Cultured Hamster Trachea. L. J. SCHIFF*, A. M. Sheflner and J. L. Graf, Life Sciences and Chemistry Division, IIT Research Institute, Chicago, IL 60616

Biological effects of in vitro exposure of hamster tracheal organ culture to carbon, benz(a)pyrene (BaP)-carbon (1:1), pyrene-carbon (1:1), or BaP-pyrene-carbon (0.5:0.5:1) were studied by light microscopy and incorporation of [3H]-thymidine as measured by autoradiography. The particles were suspended in medium and added to tracheal explants for 2 hr. Tracheal explants exposed to the three test particles at 500 µg/ml underwent necrosis after 7 days in culture. Similar changes in treated explants were observed after exposure to 50 µg/ml; however, it took 7 days longer for these changes to take place. Cultures treated with BaP-carbon or BaP-pyrene-carbon at 10 µg/ml showed alterations after 5 days, as evidenced by stratified metaplasia and cellular atypia. Peak response to these two mixtures was reached 14 days after application of the mixtures and consisted of a typical epidermoid metaplasia and slight basal cell hyperplasia. Explants treated with pyrene-carbon, carbon alone or culture controls maintained an epithelium consisting of flattened columnar cells. Thymidine-labeling index of BaP-carbon and BaP-pyrene-carbon at 10 µg/ml was comparable to that of control cultures at day 1 and then increased. By 4 days, the labeling index of BaP-pyrene-carbon-treated explants increased 2-fold above BaP-carbon values and approximately 100-fold above that of controls. Thus a synergistic effect of polycyclic aromatic hydrocarbons and particulates on respiratory mucosa was observed.

16. Selenium-Modified Metabolism and Mutagenicity of Benz(a)pyrene. R. W. TEEL* and S. R. Kain, Department of Physiology, School of Medicine, Loma Linda University, Loma Linda, CA 92350

Using Arochlor 1254-induced enzymes in the 9000 x G supernatant of homogenized rat liver as the metabolic activation system we have found that both the metabolism and mutagenicity of benz(a)pyrene is altered in the presence of selenium. Selenium inhibited the mutagenic effects of benz(a)pyrene on Salmonella typhimurium strain TA100 and of the following metabolites of benz(a)pyrene: 7,8 and 9,10-diol, and 3-hydroxy when these metabolites were used as substrate. Spectrophotofluorometric measurements of aryl hydrocarbon hydroxylase (AHH) activity in the metabolic activation system indicated that AHH was inhibited by selenium. This was further supported by high performance liquid chromatographic (HPLC) analysis of benz(a)pyrene metabolites in which selenium in the metabolic activation system significantly inhibited the formation of 3-hydroxy, 7,8- and 9,10-diol, 1,6- and 3,6-dione. When used as the substrate the amount of unmetabolized 3-hydroxy was threefold greater in the presence of selenium as determined by HPLC. Isolation of conjugated metabolites by eluting samples on an alumina column indicated that the amount of water-soluble metabolites was decreased but the amount of unmetabolized benz(a)pyrene and unbound metabolites was increased in the presence of selenium. These observations suggest that selenium modifies the metabolism of
benz(a)pyrene in a way that decreases mutagenesis and carcinogenesis.

17. Inhibition of DNA Synthesis Following Ultraviolet Irradiation and N-Ethyl-N-Nitrosourea Treatment of Various Human Cell Types in Culture. R. E. GIBSON-D'AMBROSIO*, Y. Leong and S. M. D'Ambrosio, Department of Radiology, College of Medicine, Ohio State University, Columbus, OH 43210

Cell cultures of human fetal brain, intestine, kidney, lung and skin were studied to determine the effect of ultraviolet light (UV) and N-ethylnitrosourea (ENU) on normal DNA synthesis (NDS). Cultures of skin and lung fibroblasts were established and maintained in modified MEM containing 10% Fetal Bovine Serum (FBS), buffered with 15 mM HEPES. Brain cells cultures were established and maintained in the above modified MEM containing transferrin (2 µg/ml) and insulin (5 µg/ml). Epithelial-like cultures from lung, kidney and intestine were established and maintained in a modified Alpha MEM. Under these conditions, cell lines exceeded 10 (brain), 20 (intestinal, lung epithelial) and 30 (kidney epithelial) population doublings (PDL). Inhibition of NDS was compared in cell cultures less than 10 PDL. The level of NDS inhibition following UV irradiation ranged from 60% to 90% depending upon cell type and dose. Recovery from this inhibition was not observed in any of the cell types 4 hours after treatment with 5J, 10J and 20J of UV. The NDS inhibition patterns after treatment with 1, 2.5 and 5 mM ENU were also similar. Treatment with ENU resulted in an initial inhibition of 60% to 80%. Approximately 20–40% of the ENU induced inhibition was recovered 2–3 hours post-treatment. In all cases studied no signs of total recovery of NDS was observed after either UV or ENU within the 4 hour time studied. Supported by USEPA CR-807268.

18. Metabolism, Binding to DNA and DNA Repair Following 7,12-dimethylbenz[a]anthracene Treatment of Human Lymphocytes In Vitro. S. M. D'AMBROSIO, C. T. Oravec and D. New (Sponsored by R. E. D'Ambrosio), Department of Radiology, College of Medicine, The Ohio State University, Columbus, OH 43210

Human lymphocytes were obtained from normal individuals and treated in culture with 7,12-dimethylbenz[a]anthracene (DMBA). The rate of metabolism was measured over a 24 hour period using 0.5 µM [3H]DMBA. Overall 14–24% of the DMBA was converted to water and organic soluble metabolites. Of these 35–63% were conjugated products, i.e. glucuronides and sulfates, and the remainder were Phase I metabolites. Following HPLC, we observed very small amounts of phenols, 7-OH-DMBA and 7-methyl-12-OH-DMBA. Significant amounts of 7,12-DiOHmbA and DMBA-8,9-dihydrodiol were observed. The level of DMBA binding to cellular DNA was less than 5 μmoles per mole DNA-P. DNA repair was measured by unscheduled DNA synthesis in the presence and absence of S-9 rat liver microsomes. A time dependent increase in UDS was observed after a 5 hour incubation period with 1.0 µM DMBA. Significantly more (4-fold) UDS was observed with S-9. Upon incubation of the lymphocytes with DMBA for 24 hours and at higher concentrations, significant levels of UDS were induced without S-9. These data are consistent with the level of metabolism DNA binding observed. Our studies indicate that human lymphocytes in culture have the capacity to metabolize DMBA and repair subsequent DNA damage induced by DMBA. Supported by USEPA CR-807268.

19. Comparison of Rat Ventral Prostate Cell Lines Established by Exposure to Cadmium Chloride and Herpes Simplex Type 2 Virus. L. TERRACIÒ* and M. Nachigal, Departments of Anatomy and Pathology, University of South Carolina, Columbia, SC 29208

Cadmium and herpes simplex virus type 2 (HSV-2) have been implicated in adenocarcinoma of the prostate. In this study we have compared rat ventral prostate (RVP) cell lines established by exposure to cadmium chloride (CdCl2), HSV-2, and CdCl2 and HSV-2. RVP epithelial cell cultures were established (Anat. Rec. 197: 239, 1980) and treated with CdCl2 (10-4 – 10-5 M), HSV-2 (333 strain) inactivated by UV irradiation for 40–640 seconds (1.2 x 105 – 3.5 x 106 ergs) or CdCl2 followed by HSV-2. Each of these treatments resulted in cell lysis. The cell lines resulting from exposure to CdCl2 or HSV-2 alone exhibited a fibroblastoid morphology by light and electron microscopy and had a hyperdiploid chromosome number (43–45). The control cell lines which were not exposed to either agent were also fibroblastoid but had a normal diploid karyotype (42). The cell lines established from the combined CdCl2 and HSV-2 treatment had an epithelial morphology and possessed a hypotetraploid karyotype (82). These cells grew and formed epithelial islands characteristic of RVP epithelial cells in vitro. Electron microscopy indicated that the cells were joined by desmosomes and tight junctions and contained protein synthetic organelles and secretory granules. The cell lines treated with HSV-2 alone possessed immunohistochemically detectable viral antigen and exhibited resistance to HSV-2. The cell lines treated with CdCl2 were resistant to CdCl2. The cell lines developed from the combined treatment possessed viral antigen and were both CdCl2 and HSV-2 resistant. The results indicate that both CdCl2 and HSV-2 are necessary to establish transformed RVP epithelial cell lines in vitro. (Supported by a USC American Cancer Society Grant.)

20. Constitutive Δ5,3β-hydroxysteroid dehydrogenase activity in Kirsten murine sarcoma virus (KiMSV) transformed adrenal cells: possible activation of an oncotic gene. N. AUERSPERG* and K. Farcnik, Anatomy Dept., University of British Columbia, Vancouver, B.C., Canada V6T 1W5

Eight transformed tumorigenic cell lines were obtained by KiMSV infection of myofibroblast-like cells in passage 1, derived from adult rat adrenal cortex. All lines expressed Δ5,3βHSD activity, which is a differentiation marker for steroidogenic cells. Δ5,3βHSD was not detectable in the myofibroblastic target cells nor in any other adrenocortical cell type under the culture conditions used to propagate the transformed lines (continuous proliferation in medium with 10% FBS; no ACTH or other hormone supplements). To determine
whether the constitutive $\Delta\delta$HSD activity of the transformed cells was due to the activation of an oncofetal gene in fibroblasts derived from the adrenal connective tissue capsule, cryostat sections of rat embryos, 14 d postconception (PC) to birth, and of adrenal glands on days 1–6, 44 and 90 after birth were tested histochemically for $\Delta\delta$HSD. The same or adjacent sections were stained for PAS-positive material and reticulin, and with H&E. Weak but definite $\Delta\delta$HSD activity overlapped with fibroblast-like cells and connective tissue components in the periphery of the glands from day 17 PC to day 6 after birth. Subsequently, $\Delta\delta$HSD activity over the capsule diminished and was absent in the adult. The results support the hypothesis that embryonic adrenocortical fibroblasts transiently express a steroidogenic phenotype which is reexpressed in transformed cells.

21. Retinoid Reversal of Squamous Metaplasia in Organ Cultures of Tracheas Derived from Hamsters Fed Vitamin A-Deficient Diet. D. P. CHOPRA*, Southern Research Institute, Birmingham, AL 35235 Cytochemical and ultrastructural studies were carried out to elucidate mechanisms involved in the reversal of squamous metaplasia (SM) by $\beta$-retinoic acid in organ cultures of tracheas derived from vitamin A-deficient hamsters. Tracheal cultures exhibiting focal areas of SM were treated with the retinoid for up to 7 days. The retinoid significantly inhibited $^3H$-thymidine-labeling indices in the basal cells and stimulated the labeling indices in mucous cells. At the ultrastructural level, the retinoid induced marked remodeling alterations in the metaplastic epithelium that included (a) disruption of desmosomes and widening of intercellular spaces, (b) extensive vacuolation and degeneration of the metaplastic cells, (c) extrusion of the degenerated cells, (d) aggregation of keratin filaments, and (e) differentiation of certain basal cells into secretory cells. Consequently, most degenerated metaplastic cells were extruded and the epithelium repopulated as a result of differentiation of basal cells into mucous cells and hyperplasia of the preexisting mucous cells. The degenerative effects of the retinoid were limited to the metaplastic foci since the uninvolved epithelium adjoining metaplastic foci were not significantly altered.

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22. Sister Chromatid Exchange (SCE) Response to Tumor Promoting Agent (TPA) in Fibroblasts Predisposed to Malignancy. S. L. WENGER* and M. W. Steele*, Department of Pediatrics, School of Medicine, University of Pittsburgh and Children's Hospital of Pittsburgh, Pitts., PA 15213 SCE is a widely used assay for rapid detection of potential mutagenic and carcinogenic agents in vitro. Also, cultured lymphocytes from patients with certain malignancies have shown increased SCE. An 11-fold increase in SCE is seen in Bloom Syndrome (BS) — an autosomal recessive disorder predisposing malignancy. One general mechanism for malignancy is hypothesized as a two-step process: 1) initiation — the genetic phase where DNA is altered and 2) promotion — the cellular change which expresses the phenotypic phenomenon termed malignancy. To evaluate initiation, cultured normal and BS fibroblasts were treated with 1$\mu$g/ml of 12-o-tetradecanoyl-phorbol-13-acetate (TPA). Treated normal cells showed no increase in SCE whereas treated BS had an increase in SCE (p<0.05). To evaluate promotion, the replication rates of the cultured cells were examined using differential staining of metaphase plates after 72 hr. BUdR incorporation. A large proportion of first division cells vs. second division cells were seen after TPA exposure for both normal and BS cultures and similar mitotic indices were noted for both cell lines. These two data suggest that TPA delays cell division equally in both normal and BS cells. Therefore, our results suggest that the propensity for malignancy in BS may be within the initiator rather than promotor step. (Supported in part by BRSG S07-RR20 and ACS IN-58S).

23. Cytogenetic Studies with an Opossum Cell Line (Didelphys virginiana). ASIT B. MUKHERJEE* and Diane Esposito, Department of Biological Sciences, Fordham University, Bronx, NY 10458 Cytogenetic studies were performed on an established cell line (OK) derived from the kidney tissue of Didelphys virginiana (2n=24). This line was shown to have a modal number of 23, and a notable degree of aneuploidy. Karyotypes exhibited 11 sets of pairable chromosomes and one nearly metacentric marker chromosome. The C-banding pattern revealed the exclusive localization of constitutive heterochromatin within the sex chromosomes consistent with previous reports. With our modified techniques, G-banding patterns enabled us to clearly identify all of the OK chromosomes, in contrast to previous reports. The large marker chromosome was identified to be the result of fusion between two telocentric chromosomes, nos. 8 and 9. Our G-bands were consistent and histograms were constructed without difficulty. Q-banding methods, while producing some degree of longitudinal differentiation in the chromosomes, did not produce consistent results. Spontaneous sister chromatid exchange (SCE) frequency of the chromosomes is quite similar to that of eutherian mammalian species. The frequency of the chromosomes is quite similar to that of eutherian mammalian species. The frequency of induced SCEs as a result of exposure to 4 alkylating agents (EMS, MMS, ENU, and MNU) increased with increasing dosage for all 4 agents. The frequency of SCEs was not significantly different between the two telocentric chromosomes, nos. 8 and 9. Our G-bands were consistent and histograms were constructed without difficulty. Q-banding methods, while producing some degree of longitudinal differentiation in the chromosomes, did not produce consistent results. Spontaneous sister chromatid exchange (SCE) frequency of the chromosomes is quite similar to that of eutherian mammalian species. The frequency of the chromosomes is quite similar to that of eutherian mammalian species. The frequency of induced SCEs as a result of exposure to 4 alkylating agents (EMS, MMS, ENU, and MNU) increased with increasing dosage for all 4 agents. The methyl derivative of the alkane sulfonates and methyl derivatives of the nitrosamides were more potent inducers of SCEs than their ethyl analogs.

(Supported by a grant from the Whitehall Foundation, Inc. awarded to A. M.)

24. Electrophoretic Variation in Human Cells Transfected with a Mouse Adenine Phosphoribosyl Transferase (APRT) Gene. J. TISCHFIELD,* P. Stambrook, T. Oblak and J. Trill, Medical College of Georgia, Augusta, GA 30912 APRT human HT1080 fibrosarcoma (HTD-114) and Chinese hamster ovary (D50-11) cells were transfected with either plasmids or whole lambda phage containing a cloned mouse APRT gene. Sixty APRT+ transfectants were selected, at an average frequency of $8\times10^{-3}$, in
medium containing alanosine, azaserine and adenine. Starch gel electrophoresis of transfectants demonstrated a single band of APRT activity comigrating with normal mouse activity (type I). An exceptional human cell transfectant (MAPRT-2) exhibited type 1 APRT plus an equally intense and well separated band with substantially slower migration (type 2). Subclones of MAPRT-2 showed either type 1, type 2 or both types of APRT. That some subclones showed only type 1 (wild-type mouse) APRT. That some subclones showed only type 1 (wild-type mouse) APRT, having lost the novel type 2, argues against type 2 originating from a second site reversion within the endogenous defective human APRT gene. We are testing, via restriction endonuclease analysis, whether type 2 APRT is derived from an RNA or protein processing error, a mutant mouse gene or a re-combinational event between the endogenous defective human gene and a transfected mouse gene. Individual transfectant clones show great differences in stability. Studies of the unstable transgenome suggest that it can be physically lost or, in some instances, reversibly inactivated. (Supported by NSF PCM-8118283).

25. A Reproducible Method for Establishment of Clonal Epithelial Cell Lines From Normal Adult Rat Liver. K. FURUKAWA*, T. Shimada*, Y. Mochizuki and G. M. Williams*, American Health Foundation (K. F., T. S., G. M. W.), Valhalla, NY 10595, and Sapporo Medical College (Y. M.), Sapporo, Japan

We have developed a highly reproducible method for the establishment of epithelial cell lines from adult rat liver. Following a sequential perfusion with collagenase and dispase, dissociated cells are cultured in Williams' Medium E supplemented with heat-inactivated fetal bovine serum and dexamethasone. The clonal growth of these cells was detected on day 3-6, and after 7 days, the cells continued to grow in the hormone-free media without changes in epithelial appearance. These epithelial cells appear to be derived from small cells which remain in the supernatant after centrifugation at 50 g for 1 min and are sedimented by 5 min, while more than 90% of hepatocytes are sedimented by 1 min. Two of 6 cell lines established by this method formed tumors in newborn rats. All of these tumors were typical adenocarcinomas in which the epithelium was arranged in ductal structures with γ-glutamyltranspeptidase activity, suggesting the origin of cells other than mature hepatocytes.

26. Some Properties of New Clonal Epithelial Cell Lines Established from Adult Rat Liver. T. SHIMADA*, K. Furukawa* and G. M. Williams*, Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, NY 10595

Properties of 6 new clonal epithelial cell lines which have been established from adult rat liver by sequential perfusion with collagenase and dispase were examined. At passage 12, none of these 6 lines grew in soft agar but 2 lines possessed high gamma-glutamyltranspeptidase (GGT) activity and low colony forming efficiency (CFE) in low Ca++ medium. Four other lines, however, had low GGT activity and high CFE in low Ca++ medium. At passage 24, the first 2 lines produced adenocarcinoma in newborn syngenic rats with low CFE in soft agar. Therefore increased GGT activity and decreased CFE in low Ca++ medium may serve as early transformation markers of liver epithelial cells established by this method. One of two transforming lines demonstrated responsiveness to liver tumor promoter phenobarbital by showing increased CFE in soft agar at passage 20. These lines have potential for use in transformation and promotion studies.

27. Isozyme and Chromosome Data Base of Human Cell Lines. M. J. OTTENREIT, B. Hukku, M. Mally, and W. D. Peterson, Jr.*, The Child Research Center, Department of Pediatrics, Children's Hospital of Michigan and Wayne State University School of Medicine, Detroit, MI 48201

Our laboratory characterizes cell lines by utilizing isozyme and chromosome analysis. The results are entered into a computer data base, along with results from other laboratories. The data base is used to help answer questions concerning cell line authenticity. Data on the frequency of isozyme phenotypes (G-6-PD, PGMI, PGM3, ESD, Me-2, AK-1, and GLO-1) in the human tumor lines have been compared with phenotype frequencies in the normal population. Of the cell lines we have analyzed, only G-6-PD, ESD and AK-1 phenotypes showed normal population frequencies. The remainder of isozyme phenotypes differ significantly in frequency from the normal, a finding that corroborates results of Wright et al (JNCI 66: 240, 1981). Six percent of cell lines had the most common isoenzyme phenotype. Much lower percentages had identical isozyme profiles when one or more of the less common phenotypes were present. Cell lines with the same isozyme profiles were distinguished with the aid of a data base on chromosomal characteristics. The linkage between the chromosomal and isozyme data bases provides a valuable tool for correlating findings on cell lines submitted for characterization. Supported by NCI Contract 3N01-CP-21017

28. Ultrastructural Study of the Development of Rat Myocytes in Monolayer Culture. L. R. PAPA*, G. M. Ciraco, and P. T. McCauley, Toxicology & Microbiology Division, HERL, USEPA, Cincinnati, OH 45268

The aim of the study was to investigate structural changes in cultured beating heart cells and to establish a time course in development. Heart tissue from 3-day-old rats was dissociated by trypsinization. Isolated cells were cultured and maintained for 20 days in EMEM supplemented with 5% FBS. Cells were fixed in 2% glutaraldehyde, dehydrated in a series of 30% thru 100% EtOH solutions and then embedded in TAAB. Grids were stained with 2% uranyl acetate and 0.5% lead citrate. The electron micrographs showed that in all cells myofilaments were present. In some cells, the myofibrils appeared to be randomly organized throughout the cytoplasm and incompletely developed. In those early cells in which highly developed myofibrils did appear, development was occurring along the periphery of the cell. Older cells contained well defined membrane systems, T, A, and Z bands were clearly visible throughout the cell, and
myofibrillar bundles were highly organized and branch-
ing. A T-tubular system appeared to be present only in older cultures and was restricted to the region of the Z band. Although it is evident that maturation is occurring at different rates within individual cells, there are defin-
ite stages separating early and late cultures. We feel this technique could be useful in assessing toxicity in cul-
tured cells. (This is an abstract of a proposed presenta-
tion and does not necessarily reflect EPA policy).

30. Effects of Sodium Butyrate on Adult Rat Hepatocyte Cell Cultures. G. L. ENGELMANN*, J. L. Steacker*, A. Richardson* and J. A. Fierer*, ‘Pathology Department, University of Illinois College of Medicine at Peoria, Peoria, IL 61656
Sodium butyrate, at millimolar concentrations, appears to mediate/initiate multiple effects on many mammalian cells in culture. Preliminary studies using freshly iso-
lated hepatocytes showed that 5 mM butyrate decreased RNA synthesis without affecting protein synthesis. To study the effects of long term exposure to this agent, serum-free primary cell cultures of adult rat hepatocytes were established and which maintained many adult char-
acteristics during a four day culture period. Constant ex-
posure to 5 mM butyrate did not effect cellular viability or morphology. Butyrate exposure retarded the progres-
sive decline in cytochrome P-450 levels and 5'-nucleot-
idase activity. The increase in alkaline phosphatase ac-
tivity was reduced to 50% of untreated cultures. There was no effect on the basal level of tyrosine aminotrans-
ferase yet butyrate inhibited glucocorticoid induction. Butyrate had no effect on either tryptophan pyrrolase specific activity or percentage of heme saturation. Gamma-glutamyl transpeptidase activity does not in-
crease during the four day culture period and butyrate had no inductive properties on this fetal characteristic. Increase exposure periods to butyrate are currently being investigated.

31. Effect of Selenious Acid on Microsomal Cyto-
chrome P-450 Content of Primary Cultures of Adult Rat Hepatocytes. G. L. ENGELMANN* and J. A. Fierer, Pathology Department, University of Illinois College of Medicine at Peoria, Peoria, IL 61656
Serum-free cultures of adult rat hepatocytes demonstrate progressive reductions in the microsomal cytochrome P-
450 (P-450) content during the first 24–48 h in vitro. Many media modifications have been formulated to main-
tain in vivo levels of P-450 during longer culture periods. Prior studies have shown that exogenous heme helps to retain near in vivo levels of P-450 for 48–72 h. Using this system, conventional phenobarbital (PB) mediated induction of P-450 was not attained. With the addition of 0.1 µM selenious acid to the heme containing medium, PB mediated increases in microsomal P-450 levels were observed. After 48 h exposure to 1 mM PB, P-450 levels increased to 196% of the level found in freshly isolated hepatocytes. Increases in P-450 content were also obtained by treating the hepatocytes with the immunomodulating agent levamisole. The selenious acid slowed yet did not eliminate the decline in cytochrome b, levels normally observed. Selenious acid had no effect on cellular viability or morphology during a four day cul-
ture period. This report substantiates the observation of Newman and Guzelian that adequate selenium concen-
trations are necessary for in vitro P-450 induction.

32. Morphological and Functional Differentiation of Individual Hypothalamic Nuclei in Vitro. A. T. GYÉVAI*, G. B. Makara, E. Stark and M. Palkovits, Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1450 Budapest, Pl. 67, Hungary
A special technique developed by Palkovits allowed us to isolate individual hypothalamic nuclei from 17–18 day old rat embryos and follow their morphological differ-
entiation as a function of time in vitro by electron microscopy. We found that hypothalamic nuclei have a capacity of self-differentiation. The nucleus praeopticus, nucleus paraventricularis and nucleus posterior, how-
ever, failed to develop an adult-like morphological pat-
tern in cultures. The nucleus praeopticus, nucleus para-
ventricularis, nucleus periventricularis and nucleus arcuatus maintained for 4 weeks in vitro released corticotropin-releasing-hormone spontaneously. There was no activity in the media decanted from the nucleus dorsomedialis and nucleus ventromedialis. Furthermore all of the nuclei which were cultured released a substan-
tial amount of hormones which altered the basal STH secretion in the monolayer cultures prepared from adult rat hypophysis. SRIF activity appeared earlier in the media decanted from the nucleus ventromedialis than in the media decanted from the other nuclei.
33. Single Cell Variability in Serum Albumin mRNA in Rat Hepatoma Cells. J. A. Peterson, J. M. Taylor, J. W. McLean, and W. L. Chaovapong. *Bruce Lyon Memorial Research Laboratory, Children’s Hospital, 51st and Grove Sts., Oakland, CA 94609; 2GladeSt Foundation Laboratories, 2550 23rd St., San Francisco, CA 94110

We have analyzed the molecular basis of the clonal variability in serum albumin synthesis in rat hepatoma cells at the single cell level. A clonal rat hepatoma cell line (Fu5-3B) synthesized albumin at a constant rate over many cell generations. However, the albumin content in single cells detected by immunoperoxidase techniques showed extensive quantitative variability over a 10-fold range. This single cell diversity was generated very rapidly, since clonal colonies of less than 40 cells exhibited almost as much variability as the entire population. Using a 1H-labeled cDNA probe, prepared from an M-13 recombinant phage containing albumin mRNA coding sequences, with in situ hybridization techniques, we demonstrate a 10-fold range of variability in albumin mRNA content per single cell, as quantitated by grain counts. Normal Rat Kidney (NRK) cells that do not synthesize albumin show no in situ hybridization with the albumin cDNA probe. These results suggest that the variability in albumin synthesis is at the level of albumin mRNA synthesis, presumably either mRNA processing or transcription.

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34. Growth Requirements and Differentiated Functions of Cultured Human Urothelial Cells. T. M. O’Connell and J. G. Rheinwald*, Dana-Farber Cancer Institute and Department of Physiology and Biophysics, Harvard Medical School, Boston, MA 02115

Urothelial (urinary tract transitional epithelial) cells can be cultured from human fetal and young adult urinary bladder using a 3T3 feeder layer and an improved keratinocyte medium. Urothelial cells grow with an unstratified, closely packed morphology, and tend to elongate and form whorls in large colonies. Their growth requirements are very similar, but not identical, to those of keratinocytes—a 3T3 fibroblast feeder layer and hydrocortisone are essential, and insulin is strongly stimulatory for clonal growth in DME/F12 + 5% fetal bovine serum. For urothelial cells, however, cholera toxin (CT) and high adenine (ade) concentrations are essential, while EGF is absolutely essential. Adult hepatocytes maintained in culture progressively substitute for the fetal forms throughout the days of culture, the fetal (bound) forms. Hepatocytes cultured in standard conditions show an increase of both fetal and adult forms. Dexamethasone (Dex) prevents this ontogenic reversion and maintains many biochemical functions over many cell generations. Some controversial results have been reported concerning the effect of Dex on GGT activity. While in our hands (1) Dex blocked the rise of GGT, other authors (2) found an induction of GGT by glucocorticoid. We have examined the effect of Dex on GGT isozyme pattern in culture hepatocytes. Two forms of the enzyme could be isolated by chromatography on ConA-Sepharose: the fetal (unbound) and the adult (bound) forms. Hepatocytes cultured in standard conditions showed an increase of both fetal and adult forms throughout the days of culture, the adult form being progressively substituted by the fetal form. On the 8th day of culture 35-40% of total activity remained unbound to the lectin. However, when Dex was present in the culture medium the lower GGT activity, the percentage of the enzyme that remained unbound to the lectin was very close to that reported in adult liver (10%). We conclude that the presence of Dex in the culture medium avoids not only the rise of total
38. Culture and Characterization of Normal, Pre-tissues, tumors (Tu) and "preneoplastic" (PN) tissues

Two types of PN tissue were obtained from humans: ab- or PN tissue. The latter appeared pathologically normal. With dlmethylhydrazine (DMH) were the source of Tu were harvested from rat and human colons, and grown in vitro. In the rat studies, animals treated for 16 weeks showed intermediate results. Primitive tumors provided the lowest numbers. At the electron microscopic exam- ination, neuroblastoma strains colonies are composed of undifferentiated cells while primitive tumors and bone marrow metastases colonies are composed of well differ- entiated neuroblasts containing many neurosecretory granules. This method seems valuable to study the prog- nosis of the neuroblastomas and can be applied to an individual chemotherapeutic sensitivity evaluation. (Supported in part by Justine Lacoste-Beaubien Foundation, Hôpital Ste-Justine.)

39. A Kinetic Analysis of the Induction and Decay of the Antiviral State by Murine Placental Inter- feron and Type 1 Interferons. C. D. REED*, P. T. Allen, O. S. Weislow, and A. K. Fowler, National Cancer Institute, FCRF, Frederick, MD 21701

We recently reported an interferon(IFN)-like activity in placental extracts of random-bred NIH/NIH(S) mice and from several inbred strains. The placental antiviral activity (MulIFN-PL), as measured by the inhibition of virus-induced cytopathic effects in L929 cells, is partially labile at pH 2, is inactivated by 56°C/60 min or trypsin treatment and requires cellular transcription. Although MulIFN-PL activity is partially neutralized (>40%) by high concentrations of antibody to murine type 1 IFN (MulIFNa/β), it is antigenically distinct from type 1 and type 2 murine IFNs. The present study describes the kinetics of the development and decay of the antiviral state by MulIFN-PL, MulIFNa/β and human recombinant alpha IFN (HuIFNaA/D) in L929 cells. Kinetic analysis indicates that protection induced by MulIFN-PL and type 1 IFN becomes maximal in 9 hrs, but 50% protection occurs in 3 and 5 hrs with the respective IFNs. In contrast, upon their removal the antiviral ef- fects conferred on cells by these IFNs decay at strikingly different rates. The protective activity of type 1 IFNs is rapidly lost (<25% protection at 10 hrs) while MulIFN- PL protection persists (>50%) for up to 24 hrs. Inter- estingly, the induction and decay of the antiviral state by HuIFNaA/D was indistinguishable from murine type 1 IFN. These data are consistent with the hypothesis that MulIFN-PL represents a class of IFNs distinct from types 1 and 2.

40. Characterization of Infectious Mononucleosis (IM) Downey Cells, and Purification of Paul-Bunnell (PB) Antigen. A. F. JELDE*, P. Bennett, K. Roholt, R. Stelluto, P. Bennett and J. Kellam, Roswell Park Memorial Institute, 666 Elm St., Buffalo, NY 14263

Studies have further confirmed early reports concerning Paul Bunnell (PB) heterophile antigen (Ag) in 40% surgically removed spleens from lymphoma leukemia (LL) patients, and the reactive nature of the cell population seen during IM, in which PB antibody (Ab), diagnos- tic for IM, appears in high titer along with abnormal Downey cells. The role of the Ag, Ab and Downey cells is unknown. Recent work indicates that the Downey cells are chiefly an expanded suppressor/cytotoxic T lymphocyte population. Some Downey cells have characteris- tics of NK cells. We have been unable to establish cell lines producing Pb Ag from LL spleen cultures, some having produced antigen for several months. Using hydrophobic, controlled pore glass (CPG), and lectin chromatography for purification of PB Ag from spleens, we are attempting to establish hybridomas for produc- tion of PB Ab. Use of Triton X-100 is under investiga- tion as a means of PB Ag extraction. Our studies are de- signed to further clarify the role of the PB system in ini- tiating malignancy and immunological disorders, and to
42. A-V’s for Tissue Culture Laboratory Safety Training: A Report from the TCA ad hoc Committee for Laboratory Materials and Biosafety. J. M. REED* and L. LIPSCUTZ* organizers, IIT Research Institute, Chicago, IL 60616 and DNA Plant Technology, Cinnaminson, NJ 08077

Personnel orientation and training are among the primary objectives of a safety program. Before technical staff are assigned to a research or testing program, they should be thoroughly briefed as to the potential risk of test agents or materials, routes of exposure, good handling practices and special safety and emergency procedures must be readily available. Gathering such information can be very time consuming. The ad hoc Committee for Laboratory Materials and Biosafety has surveyed films and audio/visual (A-V) aids available from various sources. Those related to tissue culture laboratory procedures have been selected for presentation during the meetings. The presentation will be highlighted in the committee poster session and summarized during discussions at our round table. Our aim is to develop a package of safety training material that is readily available at minimal cost and offers the basic knowledge required to assure safe laboratory operations.

43. In Vitro Test for Toxicants. E. BORENFREUND* and O. Borroto, Laboratory Animal Res. Ctr., Rockefeller Univ., NY 10021

Concern of animal rights groups has stimulated interest in alternatives to the use of animals for biomedical studies and toxicity testing. For range-finding experiments, we have screened different types of cells in culture for their suitability as targets for the grading and ranking of a broad spectrum of toxicants including chemicals and drugs. Normal and transformed cells of human and animal origin were seeded to 24- or 96-well tissue culture trays. Test agents were added in triplicate in a wide range of four-fold dilutions 24 hrs. later. Wells were scored after 6, 24 and 48 hrs. and concentrations were narrowed in the effective ranges in subsequent tests. Toxicity was assessed using an inverted microscope. Cell lysis, contraction and detachment from the substrate, vacuolization and marked granularity as well as dye exclusion served as criteria. The highest tolerated dose (HTD) was used as endpoint. HTD values for HepG2, a human hepatoma cell line, were higher than for low passage epithelial rabbit cornea cells; those for mouse 3T3 or V79 Chinese hamster fibroblasts were intermediate and comparable. However the overall ranking of the various toxicants was similar for all cell types examined. The test was rapid and reproducible and the data served as guidelines for dosages to be used in subsequent in vitro experiments.

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44. Chromosome Analysis and Sorting by Flow Cytometry. L. S. CRAM, M. F. Bartholdi, and J. GH. Jett.

No abstract received

45. The Use of Flow Cytometry for Studies of Cellular Differentiation. D. E. SWARTZENDRUBER and J. C. Martin, The University of Colorado, Austin Bluffs Parkway, Colorado Springs, CO 80933, and The Los Alamos National Laboratory, Los Alamos, NM 87545 (Sponsored by J. M. Lehman*)

Quantitative studies of in vitro cellular differentiation are often hampered by the innate heterogeneity of the developing cells. Many biochemical analyses of such populations are necessarily averages of all of the cells, and most cytochemical immunofluorescent assays are only semiquantitative. Flow cytometry provides a means for quantitative analysis of large numbers of individual cells for a variety of morphological, biochemical and antigenic characteristics. Several of these techniques will be described, and their use for studies of teratocarcinoma, breast carcinoma and myeloma cell differentiation will include (1) the use of light scatter for measurements of size and morphology; (1) the use of nondestructive fluorescent labels for DNA and enzyme activities to
46. Immunological Methods in the Study of Cell Heterogeneity. P. K. HORAN, Smith Kline and French Laboratories, Philadelphia, PA 19101

With the advent of monoclonal antibodies came the ability to detect surface and cytoplasmic markers with specific biochemical and biologic confirmations. Coupling this method to Flow Cytometry it then became possible to evaluate the concentration of these biomolecules on a single cell basis. We shall discuss how flow cytometry in cooperation with fluorescent monoclonal antibodies can be used to describe cell heterogeneity. Furthermore, we shall outline some of the pitfalls associated with this approach.

Analysis of cell phenotype by identifying surface biochemicals is a useful procedure but does not inform us about that cells’ ability to function. For example, to identify a macrophage from a lymphocyte or a neutrophil using monoclonal antibodies does not tell us whether the macrophages identified can perform their designated functions. A series of dyes have been developed which make possible the measurement of membrane potential by flow cytometry. These dyes are being used to measure cell function. Adaptation of this methodology to other tissue culture systems will be discussed.

47. Heterogeneous Expression of Carcinoembryonic Antigen by Human Colon Carcinoma Cell Lines. R. E. CUNNINGHAM and P. D. Noguchi,* Division of Biochemistry and Biophysics, Office of Biologies, National Center for Drugs and Biologies, Bethesda, MD 20205

Carcinoembryonic antigen (CEA) remains a controversial marker for cancer with conflicting reports in the literature about its usefulness in the diagnosis of colon cancer. We used monoclonal antibodies to CEA with flow cytometry techniques to study the WiDr and LS174T human colon cancer cell lines. CEA expression was relatively low and quite heterogeneous in the WiDr cell line; CEA expression was about 400% higher in the LS174T line and was equally heterogeneous. Correlated DNA and immunofluorescence cytograms showed that CEA was equally heterogeneous throughout the cell cycle, and that the mean immunofluorescence intensity increased approximately linearly with the cell surface area. To enrich the WiDr cell line for CEA production we sorted 2% of the most intensely staining cells into sterile flasks, grew the cells into mass culture and re-sorted the brightest 2% into flasks. The resulting population had an increase in mean fluorescent intensity for CEA production of over 200% unsorted cells. The growth rates of the two cell lines were similar, suggesting that slower growth was not responsible for the increased CEA production. Correlated DNA-CEA cytograms showed that the increased CEA production was present in all parts of the cell cycle. Thus, many of the discrepancies that have been found in the literature may be due to the marked heterogeneity of CEA expression seen in some colon cancers.

48. Flow Cytometric Cell Cycle Analysis of Immune Cell Proliferation: An In Vitro Immunotoxic Screen. J. F. ANSON,* W. G. Hinon, H. Schol, J. P. Pipkin, J. L. Hudson, National Center for Toxicological Research, HFT-164, Jefferson, AR 72079. University of Michigan, Cytometry Program, Ann Arbor, MI 48109

Automated flow cytometry has gained acceptance in the clinical setting as both a diagnostic and therapeutic aid. Data depicting fluorescent DNA content changes as a function of the state of the cell are analyzed to provide information on cyokinetic response resulting from disease or treatment. Using these techniques, the Cell Biology Laboratory at NCTR has developed a murine immune cell culture system compatible with flow microfluorometric techniques to study cellular response to toxic insult. Cultured spleen cells are exposed to the mitogen Concanavalin A (Con A) or to Con A and a chemical agent. After 48 hours in culture, the cells are examined for DNA content and a statistical comparison is performed which provides information on the cell cycle kinetics of the cultures in response to stimulation. The flow cytometric data are represented by histograms showing the number of cells vs the amount of DNA. The analysis of histogram changes between samples yields a probability that a specific dose level of a compound causes alterations in the stimulated cell cycle. A known modifier, colcemid, inhibits cycling where other compounds, e.g., diethylstilbestrol, caffeine, and phenylpropanolamine modify the kinetic response of the system.

The significance of the data generated is in its predictive value, whereby a threshold level at which cell cycle perturbation occurs is assessed.

49. Detection of Nuclear Antigen by Flow Cytometry Combined with Monoclonal Antibody, D. W. SHEN, F. Real, L. Ngo, L. J. Old, P. A. Marks, and R. A. Röfkind., (Sponsored by J. Fogh)* Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, N.Y., NY 10021

p53 a nuclear antigen of 53,000 molecular weight, which has recently been found in rapidly proliferating normal cells and a variety of transformed cells, has been considered to be closely associated with the state of cell division and cell transformation. A new approach to detect the cellular level of p53 in murine erythroleukemia cells (MELC) was developed in which monoclonal antibody (HD200-47), directed against p53, was used as a probe in the indirect immunofluorescence assay, modified for flow microfluorometry (FMF). Human SV80 cells, which are known to show strong immunofluorescence, serve as a positive cell control; the supernatant of myeloma NS-1 cells, fresh α-MEM or PBS serve as negative antibody controls. MELC were fixed in 2% glutaraldehyde and fractionated according to their size and position in the cell cycle by centrifugal elutriation in hypotonic Hanks’ solution. Cell cycle position was determined by FMF after propidium iodide staining and aliquots of cells in G1, mid-S and late S/G2, were re-fixed in
methanol/acetone, washed, reacted with monoclonal anti-p53 or control media, and then with goat anti-mouse IgG-FITC to detect p53 content. It was observed that p53 content increases progressively during the cell cycle from G1 to G2. This technique provides a method for examining p53 levels in individual cells during modulation of the cell cycle and cell differentiation. 

Changes in cell reactivity to exogenous hormones as part of tissue and organ development may underlie selection of specific explants taken at the right plant development stage. Nutrients can further modulate hormone response in organs and in callus cultures, suggesting that specific sequence treatments can be understood and exploited. Light quality influences early stages of organ formation. Humidity affects cuticle development and, via transpiration, the flow of nutrients and hormones from the media. 

Nutrient-hormone interactions also lie at the center of in vitro cell differentiation; e.g., phloem and xylem. Cell differentiation must be controlled if tissue specific genes are to be displayed for selection in culture as part of biotechnology and gene splicing applications. (Supported in part by N.C. Agric. Res. Serv. Proj. 03813 and So. Forest Research Center (industry cooperative).

50. Chemosensitivity of FANFT-Induced Mouse Bladder Tumors. A. D. DEITCH* and R. de Vere White, Dept. of Urology, Columbia University, 630 W. 168 Street, New York, NY 10033

We compared the ability of viable cell counts and DNA histograms obtained by flow cytometry to distinguish between effective and ineffective chemotherapies in monolayer cultures of mouse transitional cell carcinomas. Based on in vivo studies, cisplatinum (DDP) and 5-fluorouracil (5FU) were selected as the effective and ineffective agents, respectively. A dose range of each drug was added to log growing monolayers for 1 or 24 hr. Cultures were re-fed and harvested daily by trypsinization. Viable cell counts were made and the cell suspensions were stained by propidium iodide (PI) with ribonuclease. Contrary to in vivo findings both agents proved effective in vitro, killing >85% of the initial monolayer cells by 5 days. The primary and persistent effect of 10-60 μg/ml 5FU was a shift of the major portion of the population from the G1 amount of increased PI fluorescence, indicating a block in S apparent at 24 hr for all concentrations. This preceded cell killing by 24 hr. Effective levels of DDP altered DNA histograms in one of two ways: higher does (10-100 μg/ml for 24 hr) induced only a slight increase in S plus G2, but lower doses caused striking changes. Instead of the major G1 peak and minor S plus G2 hump as seen in untreated cultures, those receiving 1 μg/ml DDP for 24 hr had nearly equal cell numbers in all cell cycle compartments and some hyper-G2 cells. Two parameter (PI vs. light scatter) histograms showed that the large cells found after treatment had reduplicated DNA. Similar findings were obtained after other alkylating and intercalating agents.

51. Control of Cell/Tissue Growth and Differentiation—Hormonal and Nutrient Aspects. R. L. MOTT,* Dept. of Botany, N.C. State University, Raleigh, NC 27650

Methods for propagation in culture differ by plant species with respect to 1) the original explant selected, 2) nutrient media composition, 3) hormone regime, and 4) environment during culture. Interaction between these factors is strong; thus methods are usually fortuitous combinations from among the many empirical trials using guidance from related species. It is time to direct attention to understanding these factors and their interactions. The literature and examples drawn from our conifer culture experience suggest useful concepts. Changes in cell reactivity to exogenous hormones as part of tissue and organ development may underlie selection of specific explants taken at the right plant development stage. Nutrients can further modulate hormone response in organs and in callus cultures, suggesting that specific sequence treatments can be understood and exploited.
manipulating phase change characteristics, particularly root and bud forming ability, will be described, illustrated and discussed.

54. Basic Research Needs and Their Application to Micropropagation Practice. B. H. McCOWN, Department of Horticulture, University of Wisconsin, Madison, WI 53706 (sponsored by P. M. Hasegawa*)

The previous speakers have demonstrated the need to supplement our understanding of a number of developmental phenomena important to micropropagation. These include the control of embryogenesis and the successful application of this technique to a greater diversity of plants, the reversal of phase state and its consequences, the control of endogenous growth cycles in shoots, and the basis for "acclimating" tissues to culture. Other research needs involve both physiological theory and practical application. There appears to be a strong antagonism between callus and shoot growth in culture and the failure to micropropagate a number of plants is a result of our inability to control prolific callusing. Although well-known for cell cultures, evidence is accumulating that the sensitivity of intact meristems to growth regulators may change over time, particularly during the early acclimation of explants to culture. "Witchesbrooming" has been observed in a number of micropropagation systems. Acclimation of microcultured tissues to non-in vitro conditions can proceed by a number of avenues for different plant species, but failure of this process is a major problem in the micropropagation of a number of plants. Intensive research on these phenomena is critical to the successful application of microculture to agriculture.

55. A Comparison of Several Gelling Agents and Concentrations on Callus Growth and Organogenesis In Vitro. Joan L. Halquist, Mary A. Hosier and Paul E. READ*, Department of Horticulture Science, 1970 Folwell Ave., Univ. of Minn., St. Paul, MN 55108

Petunia leaf piece culture was used as a model to compare various concentrations and sources of commercially available gelling agents on callus and shoot formation in vitro. Four agar products, Difco Bacto-Agar, Gibco Phytagar, Gibco Bacteriological Grade Agar, T. C. Agar from K. C. Biological, and one heteropolysaccharide gel, Kelco Gilrite, were compared at three concentrations. Concentrations of 33% lower than recommended produced greater callus and shoot production with all agents than either recommended or 33% higher than recommended concentrations 32 days after explanation date. Production of callus and shoots on media containing Gibco Bacteriological Grade Agar was inferior to the other media at all concentrations tested. Results with woody species tissue culture will be discussed in relation to the petunia results.

56. A Comparison and Evaluation of Anther Cultures Taken from Whole Plants and from de novo Flower Buds. M. P. BRIDGEN* and R. E. Veilleux, Virginia Polytechnic Institute and State University, Butcheson Hall, Blacksburg, VA 24061

The production of de novo flower buds from epidermal and subepidermal tissues of Nicotiana tabacum 'Samsun' cultured on two media was examined for both haploid and diploid plants. The percentage of explants forming flower buds was the same regardless of ploidy status of the source plant. No difference in the flower bud-forming capacity was observed on the two media (modified Murashige and Skoog medium plus \(10^{-6}\) M Indole-3-Acetic Acid and \(10^{-6}\) M Kinetin with either 3% sucrose + 0.1 mg/liter thiamine or 3% glucose + 0.4 mg/liter thiamine.)

Androgenetic potential of anthers derived from de novo flowers was compared with that of anthers from the source plants. Ploidy levels of anther-derived plants was also examined. Anthers from de novo flower buds were not more embryonic than those from source plants; the production of hypohaploids was not realized.

57. Morphogenesis in Barley Tissue Cultures. R. C. WEIGEL JR. and K. W. Hughes*, Dept. of Botany, University of Tennessee, Knoxville, TN 37996-1100

Seeds of several barley varieties were dehusked with 50% H\(_2\)SO\(_4\), rinsed, placed in aqueous CaCO\(_3\), rinsed, sterilized with 1.05% CaO, rinsed, placed in contact with 0.1N HCl, and rinsed at least three times again. Afterwards, they were transferred to 75 mm x 150 mm culture tubes containing the medium described by T. Cheng and H. Smith (Planta (Berl.) 123, 307-310 (1975)) (no plant regulators) with 0.8% agar and allowed to germinate for 9-11 days. One cm long explants consisting of the apical meristem and adjacent stem tissue were removed and transferred to the medium described above plus 10µM IAA, 15µM 2,4-D, and 1.5µM 2-iP, which were added to the autoclaved basal medium by filter sterilization. Two months later the resulting callus was transferred to the same medium without plant regulators. About three weeks after transfer, many green photosynthetic roots, a few shoots, and small portions of very white, dense callus were observed in cultures of M2 mutagenized (diethylsulfate) barley; roots and a few shoots were observed in cultures of Atlas 57 barley; roots were observed in cultures of M2 mutagenized (N\(_2\)Na) Steptoe barley; no organogenesis was observed in Himalaya barley cultures.

58. Influence of Media, Genotype, Explant Source and Subculture on Shoot Regeneration of In Vitro Broccoli. Brassica oleracea. N. E. Bieber and J. F. REYNOLDS*, The Upjohn Company, 7000 Portage Road, Kalamazoo, MI 49001

The purpose of this investigation was to increase the efficiency of the in vitro broccoli shoot regeneration system. Parameters tested included interaction of cytokinin and auxin, explant source, genotype and subculture period. In this study, the interaction of three levels of auxin, indole acetic acid (IAA), and two levels of cytokinin, benzyladenine (BA), were compared with the routinely used MS-B medium which contained IAA and kinetin, as the cytokinin. Axillary or terminal shoot explants were analyzed to determine if origin of the tissue affected
regeneration rate. Genotypes 111 and 113 were compared for shoot regeneration capacity. Finally, the effect of two subcultures on the rates, trends and forms of regeneration was examined. The two genotypes differed in their shoot regeneration ability; 111 produced more adventitious shoots but fewer axillary shoots, while 113 produced more axillary shoots but fewer adventitious shoots. BA produced more adventitious shoots than kinetin. Adventitious shoot formation increased over repeated subculture. Terminal shoot explants produced more axillary shoots than axillary shoot explants.

60. Cloning of Cassava in Liberia. J.M. LOCKARD*, R.G. Lockard, and M.A. Saqui, Central Agricultural Research Institute, Mailbag 3929, Monrovia, Liberia

In vitro plant cloning techniques can facilitate the rapid introduction of improved varieties of agricultural crops. Cassava (Manihot esculenta, also called tapioca or manioc) is the major source of calories for about 300 million people living in the tropics and is the second most important crop in Liberia. The yield of local varieties of cassava in use in Liberia is estimated to be about 7 tons/ha. In contrast, high yielding and disease resistant cultivars being distributed by the International Institute of Tropical Agriculture in Nigeria yield 20-30 tons/ha on farmers' fields and 50-60 tons/ha under experimental conditions in which they are grown with good agronomic practices but receive no fertilizer treatment. The work described here was done at the Central Agricultural Research Institute in Liberia in response to the need to propagate the few plantlets available of each improved cultivar for quick testing in the field and eventually for supply to the agriculture extension service.

61. Plant Regeneration from a Long-term Callus Line of Birdsfoot Trefoil Stored at Low (4°C) Temperature. B.R. Orshinsky and D.T. TOMES*, Crop Science, Univ. of Guelph, Guelph, Ont. N1G 2W1 and Biotechnology Research, Pioneer Hi-Bred Int., Inc., Johnston, IA 50131

Decreased plant regeneration potential and abnormal regenerated plants are often associated with long periods of callus culture which preclude their use for plant improvement. Plants were regenerated from callus line KOU750C of birdsfoot trefoil (Lotus corniculatus) from subcultures in 3 consecutive experiments exposed to 4°C storage varying from 60-150 days. Shoot regeneration declined with increasing time of 4°C storage in 2 of 3 experiments. Callus age had relatively less effect on plant regeneration, although older callus regenerated fewer plants. Variation among plants regenerated from one experiment did not appear to be related to length of subculture or 4°C storage. The number of florets on the first umbel decreased with number of subcultures. Many plants did not attach sexual maturity during the course of the experiment. Pollen stainability (ca 60%) was low and ploidy level consistent from all subcultures. Regeneration potential and ploidy appear relatively stable over a fairly long period of culture in this line of birdsfoot trefoil. Storage of callus at 4°C for less than 150 days did not appear to inhibit regeneration or other phenotypic characteristics and might be used to extend the useful life of a valuable callus line. (Financial support of Ont. MAF and National Science and Engineering Research Council of Canada are gratefully acknowledged.)

62. Regeneration of Alfalfa from Protoplast, Cell and Cultures. D.C. W. BROWN and E.M. Koehl, Ottawa Research Station, Research Branch, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6 (sponsored by W.A. Keller*)

All commercial and wild varieties of Medicago sativa and Medicago falcata tested responded in tissue culture with at least 50% of the varietal lines showing enough somatic embryogenesis capacity to recover plants. The best regeneration response was shown by varieties which contain a high level of two of the nine possible germplasm types available; M. falcata and Ladak. Best results were obtained from the creeping rooted types such as Rangelander (93% embryogenic), Rambler (62%), Kane (45%), Hendrichs (38%) and Roamer (34%) as well as E.T. Bingham's widely used research variety Regen S (67%). Embryos will form, in some varieties, directly on primary callus of cotyledon and hypocotyl tissue cultured on a modified B5-media, where as other varieties require a brief, high 2,4-D treatment followed by culture on a modified Bladys media lacking growth regulators. Protoplasts require preculture in a complex Kao's media before regenerable cell colonies can be recovered. High protoplast plating efficiencies
63. Stimulation of Somatic Embryogenesis in Cotton (Gossypium holtzchianum Andress.). J. J. FINER,* A. A. Reilley, and R. H. Smith,* Plant Sciences Department, Texas A&M University, College Station, TX 77843 High frequency embryogenesis is essential for screening media combinations to regenerate whole plants from cell cultures of selected plant species. By varying callus culture conditions, callus origin, and suspension culture composition, we have enhanced the number of somatic embryos obtained from cultures of cotton. A reduction of light intensity received by the callus from 130 μEm·s⁻¹ to 30 μEm·s⁻¹ resulted in a 6-fold increase in embryo production. The use of somatic embryo-derived callus as opposed to hypocotyl-derived callus for initiation of suspension cultures resulted in a further 10-fold increase in embryo number. With increased number of subcultures, embryogenic potential was seen to decline gradually. This could be somewhat delayed if calli with high embryogenic potentials were visually selected and preferentially subcultured. Callus cultures which produced the largest amounts of embryos were lighter green, slower growing, and more nodulated than cultures which produced fewer embryos. A further increase in embryo yield was achieved by increasing 2,4-D levels used in the suspension cultures from 0.1 mg/l to 1 mg/l. By modifying these parameters, the amount of embryos obtained from 2 g of callus increased from an average of 2.7 to over 1000.

64. Embryogenesis from Cultured Leaf Sections of Dactylis gomerata L. B. L. CONGER* and G. E. Hanning, Department of Plant and Soil Science, University of Tennessee, Knoxville, TN 37996 The role of somatic embryogenesis in plantlet formation from Gramineae tissue cultures is of great importance in establishing totipotent cell lines for future experimentation in mutant selection. A genotype has been identified in Dactylis gomerata L. (orchardgrass) which produces embryos either directly on cultured leaf segments or from leaf-derived callus. Flat half-leaf sections from the two innermost leaves of greenhouse grown plants were plated on a solid Schenk and Hildebrandt (SH) medium containing 30 μM of 3,6-dichloro-o-anisic acid (dicamba). After 3-4 weeks, leaf segments which were originally nearest in proximity to the shoot apical meristem produced embryogenic callus whereas those which were originally more distal from the meristem produced embryoids directly without an intervening callus. Embryoids germinated and grew directly into plants when placed on SH medium containing 0 or 1 μM of dicamba. When placed on SH medium with 30 μM of dicamba, the embryos produced embryogenic callus. Histological observations of leaf cross sections showed embryoids arising from leaf mesophyll cells.

65. Regeneration of NaCl-tolerant Sugarcane Plants from Callus Reinitiated from NaCl Pre-selected Plantlets. M. C. LIU* and H. S. Yeh, Taiwan Sugar Research Institute, Tainan, Taiwan 700, Republic of China The differentiated shoot-buds of sugarcane (Saccharum sp. hybrid) cv. F177 were subjected to selection pressure on 0.17%-NaCl medium for two passages. The survived plantlets were laid down on MS media with either 0.94% or 1.17% NaCl. Callus mass was reinitiated at the base of stem explants of the survived plantlets and concomitantly some shoot-buds redifferentiated from it in the presence of NaCl. The callus which initiated from the NaCl pre-selected shoots gave higher differentiation rate (40%) than that raised from the nonselected shoot (21.5%). The NaCl-tolerant shoots so selected were cultivated to largeness and transplanted into soil with added salt level, in terms of electric conductivity, at 10 millimhos/cm. After 105 days growth, the NaCl-selected plant was found to accumulate more Na⁺ and Cl⁻ ions than the normal plant without affecting its growth vigor. All these characteristics indicate that an apparent shift towards a halophytic mode of salt tolerance within the NaCl-selected plant has occurred.

66. Inhibin Production by Cultured Hamster Sertoli Cells: Changes During Regression. A. S. BERKOWITZ* AND J. J. Heindel*, Dept. of Reprod. Med. & Biol., Univ. Texas Med. Sch., Houston, TX 77030, and Dept. of Biological Sciences, Univ. Mississippi, University, MS 38677. Regulation of mammalian reproductive competence involves the synchronous interaction of several endocrine pathways. The objective of this study was to determine if testicular regression in the hamster results in changes in Sertoli cell (SC) function that are reflected by altered pituitary function. In order to determine if inhibin production is directly related to the status of the testis, SC were repeatedly dispersed and cultured from normal (LD 14:10) and regressed (optically-enucleated) hamsters. Spent media from the first 4 days of culture of the SC was used to incubate established monolayer cultures of dispersed pituitary glands removed from the same hamsters that had been testes donors. After three days of incubation, the SC spent media or control media was removed and analyzed for accumulation of LH and FSH via radioimmunoassay. SC were scraped and the total cellular protein determined. There was a dose-related (μg total SC protein) decrease in FSH (but not LH) accumulation in pituitary cultures incubated with media from both normal and regressed SC cultures, but the maximal decrease occurred at 1270 μg of protein from regressed cultures vs. 2740-3640 μg of protein from normal cultures. Pituitary cells from regressed hamsters were more sensitive than normal pituitary cell to “normal” and “regressed” inhibin. There may be both a greater production of inhibin and enhanced sensitivity to inhibin in the regressed hamster. (Supported in part by NICHD Grant 5 P50 HD08338).

67. Selection and Characterization of Rat Prostatic Adenocarcinoma Cells Which are Inducible for Alkaline Phosphatase by Retinoid. D. H. REESE*, N. L. Block, and V. A. Politano, Department of Urology, University of Miami School of Medicine, Miami, FL 33101
The molecular mechanism of retinoid action is poorly understood primarily because few retinoid-induced gene products have been identified. Recently, we have shown that retinoic acid (RA) causes the RNA- and protein-synthesis dependent induction of alkaline phosphatase (AP) in rat prostatic cells (normal and malignant) and in bladder urothelium. As a continuation of this work, we have used a low-melting temperature agarose overlay containing p-nitrophenylphosphate to detect and select clones of prostatic adenocarcinoma cells which are strongly inducible for AP by RA. One rapidly-growing and strongly-inducible clone, designated 9-1C, which has been studied in detail, has a mean population doubling time of 14 hrs. Although its growth rate was not significantly affected by RA (10 μM), it saturated at lower (40%) than normal densities in the presence of RA. This phenomenon correlated with small-angle light scatter flow cytometry measurements showing that RA-treated cells were 35–40% larger than control cells. In the 9-1C cell, which has barely-detectable levels of AP, increased enzyme activity could be detected within 2–3 hrs after the addition of 10 μM RA. AP inducibility decreased with increasing cell density. The 9-1C line has remained uniformly inducible for AP during at least 4 mos. of continuous culture. When carried in vivo, it produces poorly-differentiated prostatic adenocarcinomas.

68. The Effect of Different Sera on Short-Term Primary Culture of Isolated Alveolar Type II Cells. R. D. GREENLEAF*, Department of Anatomy and Cellular Biology, Tufts University, Boston, MA 02111

Studies of type II cells in primary culture can provide considerable information about the metabolism of this important lung cell. For culturing type II cells, however, the most commonly used serum has been fetal bovine (FBS). In order to determine if type II cells can be cultured more effectively with serum from other species, the response of isolated type II cells cultured in medium which contained FBS or serum from different species was examined. Cells were isolated from adult rat lung with elastase, enriched for type II cells on density gradients and purified to greater than 90% type II cells by differential adherence in culture. Ham’s F12 medium (plus antibiotics) with serum from one of the following was used: bovine calf (CS), horse (HSL, rabbit (RS), goat (GS) or rat (rS). Day 1 (D1) attachment efficiency (AE) was similar for FBS (t0%) and 10% charcoal-extracted FCS and the different sera gave reduced AE compared to FBS or HS. At 10%, RS, GS and rS gave reduced AE compared to FBS or HS; CS gave only slightly reduced AE compared to FBS or HS; CS gave only slightly reduced AE compared to FBS or HS. At D1, 5% CS resulted in higher AE compared to 1% or 15% CS and 1, 5 and 15% HS. By D2, however, more cells were attached to plates when 15% HS was present in the medium. These data suggest that type II cells in primary culture respond to sera from various species and that FBS, CS and HS might give comparable results for short-term culture. In addition, certain sera (i.e., HS) could be more appropriate for longer maintenance of cells; an effect which might not be apparent at earlier times in culture.

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69. Purification of a Mammary Tumor Cell Growth Factor (Estromedin) from Sheep Pituitaries. T. SAWADA, * T. Ikeda and D. A. Sirbasku, * The University of Texas Medical School, P.O. Box 20708, Houston, TX 77225

Pituitary hormones have been implicated in estrogen-responsive growth of the MTW9/PL rat mammary tumor cells in host W/Fu rats and in estrogen-responsive growth of human MCF-7 and T47D human mammary tumor cells in nude mice. Assay of the effects of the known pituitary hormones, including prolactin and growth hormone, have not shown these activities to be mitogenic for MTW9/PL and MCF-7 cells in culture. These data raised the question of whether as yet uncharacterized factors (estromedins) secreted by normal pituitaries or pituitary tumors may be involved in supporting mammary tumor growth in vivo. We report here the purification of a new mammary tumor cell growth factor from sheep pituitaries. Lyophilized pituitary powders were extracted with 0.1 M acetic acid, the supernatant heated and applied to a Sephadex G-50 column and chromatographed on G50-Sephadex equilibrated in 0.1 M acetic acid. The final active preparation from G50-Sephadex was 96.7% pure component of MW 3900 ± 200 daltons as estimated by 8 M urea SDS PAGE analysis. Approximately 10 mg of purified pituitary estromedin was obtained from 10 g of powder, and the purified factor at 100 mg/ml one-half replaced the MTW9/PL cell response to 10% fetal calf serum. Our conclusions are that the purified activity is a new growth factor for mammary cells, and that this activity is present in considerable abundance in sheep pituitaries.

(Supported by NIH grant CA26617 and ACS grants BC-255 and FRA-212.)

70. The Effect of Oral Contraceptive Steroids on the Growth Rate of Human Mammary Epithelia in Cell Culture. S. M. Longman and G. C. BUEHRING*, School of Public Health, University of California, Berkeley, CA 94720

Epidemiological studies have suggested that the synthetic estrogens and/or progestins found in oral contraceptives may promote the growth of previously transformed breast cancer cells. Testing this hypothesis in vitro, we cultured mammary cells in media supplemented with the various synthetic hormones and measured the proliferative response. Cells used came from mammary cell lines 734B, BT-20, and Hs578t, and 60 normal, nonmalignant tissue specimens. Medium was MEM D-valine supplemented with 10% charcoal-extracted FCS and the different estrogens and progestins singly and in combination. Based on dose response studies, the hormone concentrations used were 0.1 μg/ml estrogen and 1.0 μg/ml progestin. Growth curves for each specimen in each hormonal milieu were constructed from successive in situ area measurements of two or more cell patches of 0.05 mm² size or greater. The population doubling time of each patch was calculated from the logarithmic portion of its growth curve. Cell lines 734B and BT-20 plus the majority of all classifications of primary specimens were stimulated by 17-B estradiol and the synthetic estrogen ethinyl estradiol, as well as any combination of...
hormones that included ethinyl estradiol. Cell line BT-20 and most of the malignant specimens were also stimulated by four out of the five progestins tested. Cell line Hs578t was not stimulated by any of the hormones.

71. Autocrine Control of Estrogen-responsive Mammary Tumor Cell Growth. D. DANIELPOUR* and D. A. Sirbasku.* The University of Texas Medical School, P.O. Box 20708, Houston, TX 77225

We have reported previously that MTW9/PL rat mammary tumors growing in vivo have associated an estrogen-responsive growth factor activity of either serum or tumor cell origin. In this report we have established the tumor cell origin of this activity. By several criteria, we have now demonstrated that MTW9/PL cells growing in culture under serum-free conditions synthesize a potent autostimulatory factor which remains associated with the cell. Acetic acid extracts of cells contain an autostimulatory factor with a specific activity 125-fold in excess of their conditioned medium. Treatment of the cells in culture with cycloheximide causes complete loss of activity within 3 days, while cells not treated with the protein synthesis inhibitor continue (and even increase) production of growth factor. The autostimulatory activity is estrogen inducible (5 x 10^-7 M estradiol) in serum-free medium, appears to be an acid and heat stable, 4,100 dalton MW peptide, which can be obtained in mg amounts in an approximately 50% pure state by a rapid four step purification procedure. Purity was estimated by 8 M urea + 0.1% SDS PAGE. Our conclusions are that MTW9/PL cells in culture produce an estrogen-inducible autostimulatory peptide factor and that mg quantities of this activity can be purified directly from MTW9/PL tumors growing in vivo or from MTW9/PL cells growing in culture.

(Supported by NIH grant CA26617 and ACS grants BC-255 and PRA-212.)

72. Defined Medium for Normal Human Mammary Epithelial Cells. S. L. Hammond, R. G. HAM,* M. C. D. Biology, University of Colorado, Boulder, CO 80309, and M. R. STAMPFER, Donner Lab., Lawrence Berkeley Lab., Berkeley, CA 94720

Normal human mammary epithelial cells can be grown for 3-4 passages with 1:10 splits in a complex, serum-containing, conditioned medium system developed by Stampfer et al. (In Vitro 16: 415, 1980). We have developed a defined medium system that supports better clonal growth and equivalent serum subculture without the need for conditioning or serum. The basal nutrient medium used for this growth is MCDB 170, which is derived from MCDB 202 (McKeehan and Ham, Develop. Biol. Stand. 37: 97, 1977) by decreasing cysteine to 0.07 mM, and by increasing glutamine to 2.0 mM, sodium pyruvate to 1.0 mM, and ZnSO4 to 0.5 μM. The defined supplements are EGF 25 ng/ml, insulin 5.0 μg/ml, ovine prolactin 5.0 μg/ml, hydrocortisone 0.5 μg/ml, phosphoethanolamine and ethanolamine 0.1 mM each, and prostaglandin E2, 25 nM. The defined medium supports formation of large colonies from an inoculum of 500 cells/60 mm petri dish. Primary cultures initiated from organoids in the defined medium multiply rapidly and form confluent monolayers in 7-10 days, and can be subcultured for 3-4 passages with 1:10 splits. In a similar medium without prolactin or PGE2, supplemented with whole bovine pituitary extract at a protein concentration of 70 μg/ml (Lowry), we have achieved long term subculture for up to 16 passages with 1:10 splits. There is some loss of proliferative cells at third or fourth passage. Cholera toxin reduces this loss, but is not essential for the long term growth. The cells in the long term cultures appear to remain fully normal. Supported by NIH Grant CA-30028.

73. Differential Nutrient Requirements of Normal and Malignant Human Transitional Epithelium in Serum-Free Culture. B. M. BISHAI, S. Kagawa, K. S. Narayan*, D. Kirk*, Huntington Med. Res. Inst., Pasadena, CA 91101

We have compared the specific nutrient requirements of normal & neoplastic human transitional cells in serum-free cell culture. A serum-free medium, HMRI-1, was developed which supported the growth of normal adult human transitional cells in culture for 2-3 passages. HMRI-1 is medium MCDB 152 containing 0.1 mM Ca2+ & 7 additional factors (epidermal growth factor, EGF, 5 ng/ml; insulin, 5 μg/ml; cortisol, 10⁻⁷ M, transferrin, TF, 5 μg/ml; bovine pituitary extract, BPE, 1%; ethanolamine, EA and phosphoethanolamine, PEA, both at 10⁻⁴ M. A bladder carcinoma cell line (J82) also grew in HMRI-1 but behaved very differently to the normal cells. Whereas normal cells grew as attached monolayers the tumor cells grew as floating aggregates. Logarithmic growth rates were also faster for the normal cells compared to J82 (c.f. 0.47 with 0.26 population doublings/day). Addition of fetal bovine serum inhibited the normal cells but promoted both attachment and growth of J82. Omission of BPE also promoted J82 attachment with no alteration in growth rate. Normal cell growth rate was substantially reduced by omission of either BPE, EGF or TF. Omission of these 3 factors either singly or together did not affect J82 growth rate, but it did markedly reduce the growth fraction. It was concluded that the J82 cells had simpler growth requirements than the normal cells but that they could still respond to normal cell mitogens.

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74. Replicative Cultures of Normal Human Bladder Epithelial Cells in Serum-free Medium. M. E. KAIGHN*, D. Kirk*, S. Kigawa, G. Vener and S. K. Narayan*, Laboratory of Experimental Pathology, National Cancer Institute—FCRF, NIH, Frederick, MD 21701 and Huntington Memorial Research Institutes, Pasadena, CA 91101

The purpose of this project was to isolate normal human bladder epithelia for the chemical carcinogenesis studies. Primary explants were prepared from minced surgical or autopsy specimens. The nature of the cell outgrowth depended on the nutrient formulation used. After screening several formulations, HMRI-1 was found most effective for both primary explants and sub-cultures. HMRI-1 consisted of Ham's MCDB 152 with 0.1 mM Ca²⁺, epidermal growth factor (5 ng/ml), insulin (5 μg/ml),
75. Reduction of Sera Requirement in Tissue Cultures by Hormone Supplement. H. C. Chang* and O. W. Jones, University of California at San Diego, La Jolla, CA 92039

Amniotic fluid and subsequent amniotic fluid cell cultures is an important resource for prenatal genetic studies and metabolic disorders in the fetus. A short culture time for human amniotic fluid cells would be invaluable for early diagnosis. A culture medium specially designed for amniotic fluid cells has been designed to enhance primary growth of fetal cells. It is a mixture of Ham's F 12 medium and Dulbecco's modified Eagle's medium plus 10% growth factors at 4% fetal bovine serum. Replacement of fetal bovine serum with low concentrations of donor calf serum and newborn calf serum also supports vigorous primary growth of these cells. Amniotic fluid itself included at the seeding time is beneficial for most cultures. This medium preserves growth of all cell types: fibroblast-like, epithelioid-like and amniotic fluid-like, cultures. This medium preserves growth of all cell types: fibroblast-like, epithelioid-like and amniotic fluid-like, which are generally observed in standard cultures at higher sera concentrations. Amniotic fluid samples contaminated with erythrocytes usually retard cell growth but not so in this medium. The presence of larger numbers of metaphase chromosomes facilitates early prenatal analysis. However, it may provide possibilities in genetic mapping by chromosome hybridization in situ.

76. Hormonal Determinants of Vascular Smooth Muscle Cell (SMC) Growth in Serum-Free Medium. R. Weinstein*, K. Wenc, M. B. Stemerman and T. Maciag*, Depts. of Med. and Path., Dana Research Inst. of Beth Israel Hospital and Harvard Med. School, 330 Brookline Ave., Boston, MA 02215

Defined Medium B (DM-B) is a serum-free, hormone-supplemented medium which supports growth of rat SMC on human fibronectin (HFN). In 1% FBS in DME, the most active of the potential SMC mitogens tested were platelet-derived growth factor (PDGF), transferrin, and somatomedins: either pure MSA and IGF-I or boiled acid extracts of human Cohn fraction IV (CFIV). Epidermal growth factor (EGF) seemed inactive. SMC plated at 6.25 × 10^4/cm² on HFN (10 µg/cm²) in DME + 5% FBS rapidly divided, achieving a density of 29 × 10^4/cm² by day 10, or 5.54 population doublings (PD). SMC similarly plated in DM-B reached a lower density of 14 × 10^4/cm² by day 10 (4.48 PD) due to a two day lag in onset of SMC proliferation in DM-B, after which the cells doubled comparably in either growth medium. Similar cultures achieved 3.36 PD after 5 days in DM-B. Deletion of PDGF permitted 2.38 PD (p<0.01); deletion of EGF permitted 2.0 PD (p<0.01); deletion of CF IV permitted 2.45 PD (p<0.01); and deletion of Tranferrin permitted 2.63 PD (p<0.01). DM-B without EGF was less growth-promoting than DM-B without transferrin (p<0.05). These data indicate that 1) DM-B actively promotes rat aortic SMC growth in vitro; 2) EGF may contribute more significantly to rat aortic SMC growth in vitro than was appreciated in the presence of growth-limiting concentrations of FBS; 3) in a plasma-free, hormone-supplemented environment, PDGF may not be an absolute requirement for vascular SMC proliferation.

77. Expression of Major Liver Metabolic Functions in Long Term Serum-Free Rat Liver Epithelial Cell Lines. P. Padieu* and M. Chessebeuf*, INSERM U 208, Faculté de Médecine, 7 bd Jeanne d'Arc, 21033 DIJON Cedex — France

Liver cells isolated from newborn rats have been inoculated after dissociation in a serum-free medium (SFM) and without any hormone supplementation. Freshly plated liver epithelial cells resumed mitosis and growth with no Kupffer and fibroblastoid cells survival. Five biochemical markers of liver differentiation were selected: (1) 1-tyrosine aminotransferase (TAT) inducibility by dexamethasone (Dex), biosynthesis and excretion of (2) cholesterol, (3) bile acids and (4) plasma protein, (3) reducing and hydroxylating processes during progesterone metabolism. TAT was inducible by 10^-6 M dexamethasone. Basal activity was 6.6 mU/mg protein and induction ratios were between 2.0 and 1.4 during the first twenty passages. The two main physiological primary bile acids, chenodeoxycholic acid and cholic acid, were identified and quantitated by GC-MS: respectively 11.4 and 63 ng/24h/mg fresh cells, values close to in vivo figures. Three plasma proteins were excreted in the medium: albumin, transferrin and complement C3 component. Progesterone was metabolized into pregnanediol, pregnanediolone, pregnanetriol and 20a-dihydropregesterone according to in vivo liver pattern. Long term cultures of rat liver cells in SFM express major biochemical markers of liver endogenous functions. They open a new area in the study of phenotypic and genotypic expression as well as in industrial use of eukaryotes.

78. Potent Stimulation of Human Umbilical Endothelial Cell Growth by Human Diploid Fibroblasts. M. KAN, S. Sunami and I. Yamane*, Research Institute for Tuberculosis and Cancer, Tohoku Univ., Sendai, Japan 980

Human diploid fibroblasts derived from embryonic lung tissue secretes into the culture medium potent growth stimulatory factor(s) for human umbilical vein endothelial cells (HUV-EC). Serum-free medium RITC 80-7 supplemented with 10% FBS did not support significant...
HUVEC growth, but when the medium was conditioned by human diploid fibroblasts and was supplemented with 10% FBS and 33 μg/l FGF, it could support rapid cell growth (doubling time, approximately 20 hr). Proliferative lifespan of cells cultured with this conditioned medium was approximately 50 PDL. The growth stimulatory activity of conditioned medium appears to be specific for vascular endothelial cells. Preliminary biochemical analysis indicates that the activity was nondialyzable, heat labile and trypsin sensitive. The specificity and potency of this activity suggests that this may play a role in vascularization of many biological processes including wound healing, inflammation and normal development in vivo.

79. Localization of Tyrosine-Specific Protein Kinase in Anchorage Independent Cells. J. F. WHELAN and G. M. Clinton, LSUMC Dept. of Biochem., 1901 Perido St., New Orleans, LA 70112 Subcellular localization of tyrosine-specific kinases has been studied extensively only for retroviral transforming proteins in virally-transformed cells. We have compared the localization of overall tyrosine kinase activity in cells that are anchorage independent for growth. Baby hamster kidney (BHK) cells selected for growth in suspension cultures and cells that were transformed by Rous Sarcoma Virus (RSV) were determined to be anchorage independent while the monolayer cultures were determined to be anchorage dependent by their growth properties in soft agar. Localization at specific sites in the plasma membrane was investigated by measuring tyrosine kinase activity in the membrane envelope of vesicular stomatitis virus (VSV) which buds from selected sites at the cellular plasma membrane. Tyrosine kinase was high, about 70% of the total kinase, in the detergent solubilized fraction from VSV grown in the anchorage independent cells compared to only 1 to 2% tyrosine kinase in the anchorage dependent cells. However, when the tyrosine kinase levels were compared in the detergent soluble and insoluble fractions of BHK cells, activity was significantly elevated in the detergent insoluble cytoskeletal structure of the RSV-transformed cells only. These results indicate that the specific localization rather than overall levels of tyrosine kinase activity may be altered in the plasma membranes of anchorage independent cells. (Supported in part by the NSF).

80. Cytoskeletal Events During Calcium or EGF-induced Initiation of DNA Synthesis in Rat Liver Cells. S. H. H. SWIERENGA*, N. Marceau* and R. Goyette, Health and Welfare Canada, Ottawa K1A 0L2, and Centre de Recherche sur le Cancer, Université Laval, Québec G1R 2J6 Nontumorigenic rat liver cells, arrested in the G phase of their cell cycle, were induced to re-enter S phase by the addition of either 1.5 mM calcium, or epidermal growth factor (EGF). Cytoskeletal changes during the onset of DNA synthesis were analysed by indirect immunofluorescence with specific antibodies to actin, tubulin, cytokeratin, and vimentin. Cytoskeletal filaments which had become disorganized or retracted from the cell periphery during low Ca exposure became reorganized and evenly distributed throughout the cytoplasm, resulting in either a well-spread cuboidal (in response to calcium) or fusiform (in response to EGF) cell shape, before the onset of DNA synthesis, as determined autoradiographically. The presence of an anticalmodulin drug, chlorpromazine, which prevented both the calcium and EGF-induced entry of cells into S phase did not prevent cell spreading or reassembly of microtubules and microfilaments, but did prevent some reorganization of intermediate-size filaments.

(81. Optimized Medium for Clonal Growth of Human Microvascular Endothelial Cells. A. KNEDLER, R. G. Ham*, Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309 Growth of human microvascular endothelial cells in standard media requires large amounts of serum, and even then is only moderately successful. We have developed an optimized medium, designated MCDB 130, which supports clonal growth of such cells when supplemented with 2% dialyzed fetal bovine serum (dFBS). We employ the procedure of Kern et al. (Circulation II: 282 abst. 1982) to prepare endothelial cells from microvessels of omental fat. Microvascular fragments are isolated from minced fat by collagenase digestion and filtration, and separated from single cells by unit gravity sedimentation on 5% BSA. The fragments are inoculated into Medium 199 plus 10% whole fetal bovine serum (or MCDB 130 plus 10% dFBS). Trypsinized cells from confluent primary cultures are stored frozen, re-established as secondary cultures and grown to not more than 25% confluency. They are then used to inoculate clonal growth assays at 1,000 cells per 60 mm petri dish. A broad survey of media revealed that MCDB 402 (which was optimized for Swiss 3T3 cells) supported the best clonal growth with 10% dFBS. Quantitative and qualitative optimization, starting from MCDB 402, generated MCDB 130, which supported good clonal growth with as little as 2% dFBS. A dramatic improvement in growth was achieved by raising the magnesium concentration to 10.0 mM. Reduction of zinc concentration and adjustments of other trace elements also improved growth. EGF and hydrocortisone synergistically improve growth with low levels of dFBS in MCDB 130. Supported by NIH Grant CA-15305.

82. Effects of Sodium Butyrate on the Cell Cycle Progression of Rabbit Articular Chondrocytes in Culture. S. Larno, M. ADOLPHIE*, X. Ronot*, P. Lechat, Laboratoire de Pharmacologie cellulaire de l'E.P.H.E., Institut Biomédical des Cordeliers, 15 rue de l'école de médecine, 75006 PARIS, FRANCE The growth of rabbit articular chondrocytes in culture was measured in the presence of sodium butyrate (1 to 3 days), very interesting drug for studying the regulation of cell growth. The cells were derived from primary cultures of cartilaginous cells obtained from young rabbit articulations. Chondrocyte growth rate decreased with increasing concentrations of butyrate (1 to 5 mM) but
The human hepatocellular carcinoma cell line 2124, 1976, was injected subcutaneously into athymic mice by twelve weeks. On autopsy 4 (16%) had developed unexpected lung nodules. Light and transmission electron microscopy examination of these nodules confirmed the presence of cells morphologically consistent with hepatocellular carcinoma. A new cell line (NL/PLC) was established by explant culture. This cell line was cloned in agarose and subcultured according to protocols used for the parent cell line (PLC/PRF/5). Injection of this cell line into nude mice resulted in 20/23 (87%) tumor established with 5/23 metastatic to the lung, some as early as six weeks. Karyotype analysis showed that the NL/PLC and PLC/PRF/5 cell lines both had a modal chromosome number of 55. Both lines secreted hepatitis B surface antigen (HBsAg) at the same rate. Monoclonal antibodies were produced by immunizing BALB/c mice with the NL/PLC line. One antibody, N10G6, was chosen for further study. Radioimmunoassays using PLC/PRF/5 and NL/PLC cells as targets for N10G6 showed no difference in binding. FACS analysis revealed 93-95% staining of the N10G6 antibody to both the NL/PLC and the PLC/PRF/5 cells. We conclude that there are no obvious phenotypic differences between the cell lines, however, the NL/PLC cell line may show some metastatic advantage in producing tumors earlier.

83. Air Bladder III Cells: a New, Continuous Cell Line from Goldfish. T. B. SHEA, T. Yano, and E. S. Berry*, Biology Department, Northeastern University, Boston, MA 02115
A new cell line, Air Bladder-III (ABIII), was established from trypsinized, pooled air bladders of adult (20-25 cm) goldfish (Carassius auratus). ABIII cells were initially grown in Eagle's medium supplemented with 15% fetal bovine serum and were continuously grown in this medium for 15 passages after which Medium 199 with 10% FBS was used. The cell morphology is more fibroblastic in Medium 199 with 10% FBS was used. The cell morphology is more fibroblastic in Medium 199 and the cells grow more rapidly in Medium 199. The optimal growth temperature is 25°C, although the cells will grow at temperatures from 15°-30°C. The normal generation time observed is 48 hours. The karyotype is unstable: at the 70th passage, 58 and 102 chromosomes were most often observed with a range of 55-144. ABIII cells are extremely contact dependent, requiring contact of cell processes upon seeding to insure uniform culture growth. Confluent monolayers were observed to survive 3 months at 25°C without a media change and no loss of viability, and for 6 months with 50% loss of viability; viable cells were still present after 1 year without a change of media. Cells are stable to storage at -70°C in medium containing 10% DMSO. ABIII cells have been passaged 78 times over a 3 year period with no evidence of senescence. ABIII were also expected to be susceptible to infection with Goldfish Virus-1 and 2.

84. NL/PLC: A Human Hepatocellular Carcinoma Cell Line Established from Metastatic Foci of a Nude Mouse Tumor. G. MELINOFF**, A. Quinlan, T. Klug, R. Tubbs, V. R. Zurawski, Jr., Centocor, 244 Great Valley Parkway, Malvern, PA 19355 and 5 Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44106.
The human hepatocellular carcinoma cell line PLC/PRF/5 (Alexander, J. et al., S. Afr. Med. J. 50: 2124, 1976), was injected subcutaneously into athymic nude mice (BALB/c) for immunotherapy studies. Twenty-four of 25 (96%) control mice developed tumors by twelve weeks. On autopsy 4 (16%) had developed unexpected lung nodules. Light and transmission electron microscopy examination of these nodules confirmed the presence of cells morphologically consistent with hepatocellular carcinoma. A new cell line (NL/PLC) was established by explant culture. This cell line was cloned in agarose and subcultured according to protocols used for the parent cell line (PLC/PRF/5). Injection of this cell line into nude mice resulted in 20/23 (87%) tumor established with 5/23 metastatic to the lung, some as early as six weeks. Karyotype analysis showed that the NL/PLC and PLC/PRF/5 cell lines both had a modal chromosome number of 55. Both lines secreted hepatitis B surface antigen (HBsAg) at the same rate. Monoclonal antibodies were produced by immunizing BALB/c mice with the NL/PLC line. One antibody, N10G6, was chosen for further study. Radioimmunoassays using PLC/PRF/5 and NL/PLC cells as targets for N10G6 showed no difference in binding. FACS analysis revealed 93-95% staining of the N10G6 antibody to both the NL/PLC and the PLC/PRF/5 cells. We conclude that there are no obvious phenotypic differences between the cell lines, however, the NL/PLC cell line may show some metastatic advantage in producing tumors earlier.

85. Permanent Cell Lines Established from Hepatic Fibro-Granulomatous Lesions: their Relationship with Myofibroblasts and Ito Cells. R. BOROJEVIC* and A. A. Monteiro, Institut Pasteur, 69365-Lyon, France and Instituto de Quimica, da Universidade Federal, Rio de Janeiro, Brazil
Mesenchymal cells implicated in secretion of extracellular matrix in hepatic fibrosis are described as fibroblasts, myofibroblasts and fat-storing (Ito) cells. It is not clear if they represent distinct cells or stages of functional differentiation of a single cell type. In experimental murine schistosomiasis, periovular granulomas are local inflammatory and fibrotic reactions to schistosome eggs deposited in liver. Granulomas were isolated from livers of C3H mice infected with Schistosoma mansoni and cultured as explants or after cell dissociation. In all cases, after three months of cultivation, "spontaneously" transformed cells of mesenchymal type developed and grew as permanent cell lines. Their morphology was characteristic of myofibroblasts; they were anchorage dependent and secreted extracellular fibrinous matrix. Cells often accumulated in vitro fat droplets and, when reintroduced subcutaneously into C3H mice, they induced tumors rich in fat-storing cells. Viral particles of B type were observed budding on their membranes. These cell lines may represent a model of the cell type responsible for fibroplasia in liver fibrotic reactions.

(Submitted by FINEP and CNPq, Brazil).

86. Alanine Aminotransferase Activity with Unusually Low Glutamate and High Pyruvate and Alanine Requirement in the Cytosol of Human Fibroblasts. W. L. MCKEEHAN* and K. A. McKeehan, W. Alton Jones Cell Science Center, Old Barn Road, Lake Placid, NY 12946
Recently, we reported a general, sensitive and simple radiometric method to assay 2-oxoacid:aminoketo acid aminotransferases and 2-oxoacid:aminoacid aminotransferases and 2-oxoacid:2-hydroxyacid oxidoreduc-
87. Effects of cAMP on Surfactant Related Phospholipid Synthesis. WILLIAM STERN*, Sharon Pochron*, and Linda Nardone, Public Health Research Institute, N.Y., NY 10016 and Dept. of Pathology, The Catholic Medical Center of Brooklyn and Queens, Queens, NY 11421

The regulation of phosphatidylcholine synthesis in eukaryotic cells was studied using A549 cells, a continuous cell line derived from a human pulmonary adenocarcinoma. Confluent cultures of A549 cells were pre-incubated for 24 hours with 500 nM 8-bromoadenosine-3':5'-cyclic monophosphate (Br-cAMP) in serum-free Ham's F12K medium, following which incorporation of choline and acetate into lipids was assessed. The rate of [3H]acetate incorporation into phosphatidylcholine and other lipids decreased at least 50% in response to Br-cAMP but, in contrast, the rate of [Me-14C]choline incorporation into phosphatidylcholine increased 2 fold. Br-cAMP had no effect on the incorporation of [Me-14C]choline into choline phosphate, the intermediate in the Kennedy pathway that contains most of the water-soluble radioactivity. This suggests that Br-cAMP stimulates the synthesis of phosphatidylcholine from choline at a step beyond the synthesis of choline phosphate. Since the rate of [3H]acetate incorporation into phosphatidylcholine decreased in the presence of Br-cAMP, the observed increase in phosphatidylcholine synthesis may result from stimulation of turnover of pre-existing phospholipid, thus making more diglyceride substrate available for phosphatidylcholine synthesis. Alternatively, Br-cAMP may directly affect the rate of CDP-choline biosynthesis. Supported by USPHS grants: HL24630, HL29207 and HL30415.

88. New Inherited Metabolic Alteration in Synthesis of Glycoproteins and Glycosaminoglycans Demonstrated in Cultured Skin Fibroblasts. V. IONASESCU*, V. Pedrini, and C. Aschenbrenner, University Hospitals, Iowa City, IA 52242

A new inherited metabolic disease has been identified in a 28 year old female and her 5 year old daughter. Mild to moderate weakness and atrophy of facial and shoulder muscles with congenital onset and slow progression were present in both patients. Serum creatine kinase was 2x elevated. Biceps biopsy showed variability in fiber size, rare atrophic fibers, scattered sarcoplasmic vacuoles. The latter contained storage material which was weakly PAS-positive, stained metachromatically with toluidine blue and orthochromatically with alcian blue. Muscle glycogen showed low values. The storage material was also identified in skin fibroblast cultures. The latter contained also unusual cytoplasmic vacuoles with electron-dense amorphous deposits and irregular lamellar arrays within a single limiting membrane. Repeated biochemical studies of cultured fibroblasts in both patients identified excessive storage of a glucosamine containing polymer precipitable by cetyl pyridinium chloride after papain digestion of the cells. The uptake of [3H]glucosamine in cultured fibroblasts of the daughter was 3.5 greater than in the controls for both glycoproteins and glycosaminoglycans. The rate of turnover of the radioisotope was normal. The radioisotope studies in cultured fibroblasts of the mother showed normal values. These findings suggest that the genetic defect in this autosomal dominant myopathy is related to excessive synthesis of glycoproteins and glycosaminoglycans. It seems that this metabolic alteration is active only in childhood.

89. Growth and Differentiation of Rabbit Articular Chondrocytes in a Synthetic Serum Free Medium. M. ADOLPHE*, B. Froger, X. Ronot*, M. T. Corvol, N. Forest. Laboratoire de Pharmacologie cellulaire de l'E.P.H.E., Institut Biomédical des Cordeliers, 15 rue de l'école de médecine, 75006 Paris, France

A synthetic serum free medium (SSFM) was developed which was suitable for the growth and differentiation of rabbit articular chondrocytes. The cells were derived from young rabbit articulations. The cells were derived from liver, heart muscle and kidney tissue (KM values for glutamate = 6-25 mM, alanine = 10-33 mM and pyruvate = 300-600 mM). ScEso values for fibroblast particulate activity were similar to values reported for other tissues. The impact of cytosolic AT activity with such properties on glutaminolysis and the nutritional requirement for pyruvate in human fibroblasts will be discussed. (Supported by NIH Grant GM 27194.)

90. Biphasic Treatment of D-Glucose by Saturable Component in Human Cultured Cells. K.
MANAKA* and T. Manaka, Dokkyo Univ. Sch. of Medicine, Mibu, Tochigi 321-02, Japan

The kinetics of sugar uptake were studied in cultured mammalian cell lines grown in the protein- and fatty acid-free chemically defined medium. Two human cultured cells showed the biphasic uptake of D-glucose and 3-O-methyl-D-glucose; the Lineweaver-Burk plots might fit two straight lines. Both uptake systems (involved high and low affinity carriers) were inhibited competitively by cytochalasin B and non-competitively by ouabain. Negative cooperativity was indicated by Hill plots of initial velocities of uptake against the concentration of D-glucose. D-glucose binding analysis by Scatchard plots showed different dissociate constants for different cell types. Therefore, we conclude that the two human cultured cell lines employed in this study contain a single carrier component for active transport. Expression of this transport system is assumed to result from the adaptation or the selection by defined medium containing high levels of sugar.

91. Liver Phenotype Expression of Progesterone Metabolism in Rat Liver Epithelial Cell Lines Grown in a Serum-Free Medium. Laurent Fay and Jean DESGRES*, INSERM U208, Faculté de Médecine, 21033 Dijon, France

Liver epithelial cell lines from newborn rats grown in a serum-free medium (SFM) were incubated in the presence of $5 \times 10^{-4} \text{M} 4'$-4C-progesterone. The formation of eleven radioactive compounds points to metabolic pathways which depend on the presence of five enzymes in the cultured liver cells: a 5a-steroid reductase, a 3a- and a 3p-steroid oxidoreductase (the 3a- being predominant), a 20a-hydroxysteroid dehydrogenase more active on unmetabolized progesterone than on the 5a- or the 3a/3p, 5a-reduced metabolites and a 6p-steroid hydroxylase only active on the totally reduced ring A metabolites. These enzymatic activities were qualitatively and quantitatively similar to those expressed by freshly dissociated newborn hepatocytes. However, abnormal quantitative differences appeared in similar cell lines grown in a 10% serum supplemented medium. These results show the interest of SFM liver cell lines for the study of liver phenotype steroid metabolism due to their conservation and expression in the in vitro system in the same manner as observed in vivo.

92. A Serum-Free Medium for Fish Cell Cultures. E. S. BERRY* and T. B. Shea, Biology Department, Northeastern University, Boston, MA 02115

An undefined, serum-free medium (SSTS-199-4) was developed for use with fish cell cultures. This medium was based upon that previously developed by Lasfargues et al. (In Vitro 8: 494–500, 1973). The supplements added to Medium 199 were: 10$\mu$g/ml lactalbumin hydrolysate, 30$\mu$g/ml trypticase-soy broth, 50$\mu$g/ml Bactopeptone, 10$\mu$g/ml dextrose, 10$\mu$g/ml yeastolate, and 35$\mu$g/ml polyvinyl pyrodoilide. These supplements were initially combined in 100 ml of dH2O, autoclaved, and added to 5% of the final volume of the medium. In addition we added 20$\mu$g/ml filter sterilized bovine pancreatic insulin, 2 mM glutamine, and 0.1 mM nonessential amino acids. The addition of insulin was found to be unnecessary. Five fish cell lines; goldfish-derived CAR cells, Fat Head Minnow (FHM) cells, epithelioma papillosum cyprini (EPC) cells, Chinook salmon embryo (214) cells, and a new cell line from goldfish air bladders (ABIII) were all capable of growth in SSTS-199-4 at rates equivalent to cells grown in FBS. The morphology of all cell lines, except 214 cells, was identical to cells grown in EBS. The 214 cell line was observed to grow only in suspension after the 2nd passage in SSTS-199-4, although cell numbers were not changed. The other cell lines grew to confluent monolayers and remained attached to the flask. All of the cell lines were capable of long-term growth in SSTS-199-4 with no evidence of senescence up to 20 passages. CAR and ABIII cells were also demonstrated to retain the ability to replicate Goldfish Virus-2 at levels equivalent to cells in FBS.

93. Cytogenetic and Clinical Correlations in Neoplasms of the Human Nervous System. A. A. AL SAADI* and F. Latimer, William Beaumont Hospital, Royal Oak, MI 48072

Significance of specific chromosome abnormalities in the diagnosis and prognosis of human cancer have become widely accepted. Such information is meager for solid tumors. We report here studies of banded chromosomes in short term cultures of 103 human nervous system tumors (HNST). Of the 38 meningiomas (MA), 7 had no abnormalities. Monosomy and rearrangement of #22 was noted in 23 MAs, other abnormalities in the remaining 8. Loss of the Y was noted in 50% of male MAs. Of the 7 recurrent MAs, 6 had monosome 22 and other abnormalities, and 1 had a 44,XX,t(13;7) karyotype. Nine of the 11 invasive MAs had abnormal karyotypes. Five of the 35 glioblastomas (GA) had no abnormalities, however, abnormalities of #7, #22 and the Y were noted in 19–55% of the GAs. Of the 7 survivors for 2+ years, 4 had no abnormalities, 2 had loss of the Y and 1 had X monosomy. Survivors of a year or less had more severe abnormalities. Abnormalities of the X, Y, 7 and 22 were also noted in astrocytomas (AC), lymphomas and sarcomas. A primary pleomorph sarcoma was near-haploid and nullisomy 20. A grade I AC, which had no abnormalities, evolved into GA with 46,XX,-6,-8, -13, +3 mar. karyotype. Of the 4 medulloblastomas, one was hyperdiploid and had a poorer prognosis. The results suggest: 1) nonrandom involvement of chromosomes 7,22,X and Y in HNST, 2) chromosome abnormalities indicate poor prognosis, 3) chromosome analysis can be helpful in determining the stage of dedifferentiation of HNST.

94. Genotoxic Evaluation of Gossypol Acetic Acid in Chinese Hamster Ovary Cells. S. K. MAJUMDAR* and W. J. Petraiuolo, Department of Biology, Lafayette College, Easton, PA 18042

Gossypol acetic acid, an effective male antifertility compound in animals and humans was investigated to determine its genotoxic effects on Chinese hamster ovary cells (CHO) grown in culture. The cells were exposed to several concentrations of the compound ($0, 0.5, 1, 2, 4$ and $8\text{mcg/ml}$) dissolved in ethanol for various time periods. Each experiment was performed in duplicate and repeated three times. Inhibition of cell growth was noted at 2 mcg/ml, and a greater inhibitory effect and a marked cytotoxicity was observed at higher concentrations. Exposure of the cells to higher concentrations ($4$ and $8\text{mcg/ml}$) of the compound drastically reduced the
95. Species and Strain Comparisons of Genotoxic Effects from Ethyl Carbamate, Ethyl N-Hydroxy Carbamate and Vinyl Carbamate. Y. SHARIEF*, J. Campbell*, S. Leavitt*, R. Langenbach* and J. W. Allen*. Northrop Services, Inc., "U.S. Environmental Protection Agency, "National Toxicology Program, Research Triangle Park, NC 27709

Ethyl carbamate (EC) carcinogetic is not understood in terms of metabolic factors or species/strain specificities. We have evaluated genotoxic responses in various rodent species to EC and its known or suspect metabolites ethyl N-hydroxy carbamate (ENHC) and vinyl carbamate (VC). VC, being most active, increased SCE frequencies in bone cells 8-10 X baseline levels at 25-50 mg/kg. Mice showed significantly higher levels of SCE responsiveness to all of the chemicals than did rats, Chinese hamsters and Golden hamsters. SCE response in C57BL/6 mice (highly resistant to EC induced lung adenomas) and A strain mice (highly susceptible to EC induced lung adenomas) were similar. However, in vitro studies utilizing liver S9 enzyme mix alternatively obtained from the two mouse strains, with Chinese hamster V79 target cells, revealed very different genotoxicities. With A strain S9, significantly higher levels of SCE, gene mutation (HGPRT) and cytotoxicity resulted than when strain C57BL/6 S9 mix was used. These results in contrast to in vivo findings suggest that there may be important metabolic differences between the two strains of mice which bear upon strain specific susceptibilities to EC carcinogenesis. This inference, if correct, would also lend further support to the theory that VC is involved in this process.

96. Explant Culture of Shark Tissues. R. T. JONES*, E. A. Hudson and T. Sato, Department of Pathology, University of Maryland School of Medicine, Baltimore, MD 21201 and Laboratory of Experimental Pathology, National Cancer Institute-FCRF, NIH, Frederick, MD 21701

Spiny dogfish pups can be maintained in the laboratory distant from their point of capture for up to 15 months, thus allowing tissues to be obtained for in vitro studies. These embryos are copies of the mature adult with the exception that they have a yolk-sac attached to their intestine. Explants of rectal gland, kidney, intestine and stomach obtained from these sharks have been grown in Leibovitz L-15 medium supplemented with 0.1 M NaCl, 0.3 M urea, 1 μg/ml insulin, 0.1 μg/ml hydrocortisone and gentamicin sulfate (250 mg/ml) in the presence or absence of heat inactivated newborn bovine serum at 4°C in a controlled atmosphere chamber gassed with 5% CO2 in air with 100% humidity. The chamber was placed on a rocker platform and rocked 6 times per minute. Samples of the tissues were fixed as serial time intervals for morphologic study. Explants from all organs grew for up to 4 months with excellent preservation of normal structure. No difference could be ascertained among those explants grown in the presence of newborn bovine serum as compared to explants grown in L-15 medium without serum. (Supported in part by the Bressler Fund of the University of Maryland School of Medicine and the U.S. Intergovernmental Personnel Act of 1970).

97. Role of Extracellular Matrix Components in Bone Differentiation and Morphogenesis. A. H. REDDI, Mineralized Tissue Research Branch, National Institute of Dental Research, NIH, Bethesda, MD 20205

The origin and evolution of multicellular organisms were marked by the appearance of extracellular matrix (ECM). The major classes of components in the ECM are: collagens, proteoglycans and glycoproteins. While considerable interest has been focused on the role of intracellular molecules involved in differentiation, the information about ECM influences has lagged. Bone is a tissue with a vast expanse of extracellular matrix. In addition, bone exhibits considerable potential for regenerative growth, differentiation and morphogenesis in postnatal life. In view of this the extracellular matrix of bone might play a role in bone morphogenesis. We will review recent work in the laboratory showing that bone matrix has chemotactic, mitogenic and differentiation-inducing factors that are tightly associated with the extracellular matrix. In conclusion, our studies indicate that bone ECM is a repository of factors which locally govern bone differentiation and morphogenesis.

98. Analysis of Attachment and Attachment Factors of Normal and Abnormal Neural Tissue. M. F. D. NOTTER*, University of Rochester Medical Center, Department of Anatomy, Rochester, NY 14642

The ability of neural cells to utilize specific adherence factors was assessed by an in vitro attachment assay. A clone of Neuro-2a, a homogeneous mouse neuronal cell line was trypsin dispersed and seeded at 5x10^4 cells per well onto precoated cluster dishes. Attachment and spreading was assessed visually after 5 hrs. while attached cells were counted after 16 hrs. by trypsinization. Neuro-2a, in the presence or absence of fetal calf serum, did not attach to laminin or collagen coated plates or non-tissue culture plastic. Attachment as well as spreading was enhanced when plates were coated with fibronectin, Neuro-2a conditioned media, and Neuro-2a cell surface components. When a rat glial cell line, C6, glioma, was tested under similar conditions it was found that glioma cells could attach and spread quite readily on surface coated with the same factors as well as non-tissue culture plastic but not on laminin-coated plates. Normal rat fetal hypothalamic cells were seeded on simi-
larly coated dishes and attachment was assessed qualitatively after 16 hrs. None of the cells attached to laminin-coated plates while adherence was enhanced when cells were plated on the nonfibrin, collagen and poly-D-lysine coated surfaces. Indirect immunofluorescence indicated the presence of laminin and fibronectin on the surface of Neuro-2a, C, Gloma but not on fetal neural hypotalamic cells. These data indicate that normal neural tissue may utilize an undetermined factor for attachment while primitive, abnormal cells appear to use extracellular matrix proteins for attachment and spreading.

99. Regulation of Differentiation by Hormones and Extracellular Matrix. L. REID*, D. Jefferson, N. Ruiz-Opazo, Z. Gatmaitan, R. Enat, and L. Leinwand, Albert Einstein College of Medicine, Bronx, NY 10461

In studies on normal and neoplastic hepatocytes of rat and human origin, we have found that growth and tissue-specific functions are regulated in vitro by hormones and by substrata of liver extracellular matrix. Normal rat hepatocytes were maintained in long-term cultures showing no growth and expressing liver-specific functions if plated at high density into medium supplemented with serum and with particular hormones. To divide, parenchymal cells required low seeding densities, serum-free medium, a mixture of hormones (especially insulin, glucagon, and epidermal growth factor-EGF) and substrata of liver matrix depleted of growth inhibitors by salt extractions. Northern Blot analyses using cloned cDNA probes for albumin, ligandin (glutathione-S-transferase), CHO-B, and actin indicated that such functions are expressed when parenchymal cells are growing and when they are stationary, although the levels of expression usually differed under the two conditions. Neoplastic cells also responded to matrix and hormones, but their responses were qualitatively and/or quantitatively reduced from that of normal cells. In contrast to hepatocytes, hepatomas grew in serum-supplemented media, in serum-free, hormonally defined medium without EGF, and on liver and non-liver types of matrix. They also showed only transient (7-10 days) augmentation of differentiation (albumin, ligandin) when plated under conditions which gave stable, long-term (months) differentiation for normal hepatocytes.

100. Fibrin Enhanced Endothelial Cell Organization. J. V. OLANDER*, M. E. Bremer, J. C. Marasa*, J. Feder* and R. C. Kimes, Monsanto Company, 800 North Lindbergh Blvd., St. Louis, MO 63167

A variety of endothelial cells derived from both capillaries and large vessels forms capillary-like tubular structures in vitro. Electronmicroscopic examination of these structures showed them to be hollow tubes with the endothelial cells having overlapping and interdigitating cell processes typical of in vivo capillaries. Evidence was acquired which indicated that the orientation of the cells was such that the nonendothelial cell strands and the basal side was towards the lumen. These three-dimensional in vitro structures were seen to form about 3-7 days after the cells reached confluence. Recently we have observed that, in the presence of fibrin, the endothelial cells organize into structures within only 2-18 hrs after inoculation. Time lapse video tape monitoring showed the cells migrating and organiz-
cells from the culture fluid by a sieve mounted on the stirrer shaft. In such a system large amounts of plasminogen activator from Bowes melanoma cells in microcarrier culture could be produced. It will be compared with other systems described in literature.

103. Production of Human Tumor Lymphoma Virus by Large Scale Suspension Culture. D. J. Pickle, W. B. Leberherz*, G. R. Dyson, V. A. Roberts, M. E. Gustafson, J. A. Mikovits, and M. C. FLICKINGER*, NCI-Frederick Cancer Research Facility, Fermentation Program, P.O. Box B, Frederick, MD 21701

The production of human tumor lymphoma virus (HTLV) in large scale suspension culture was evaluated in three hundred liter fermentors according to P3 containment guidelines. Cultures were adapted to grow in suspension at reduced levels of serum (FBS) on RPMI 1640. These studies were extended to small scale (1.5 l) fermentor studies using a semicontinuous system. Both HTLV and constitutive human T-cell growth factor (IL-2) were produced in the reduced serum cultures. A batch production system was scaled-up to the 300 liter scale using impeller agitation and direct gas sparging for aeriation with a 5% FBS RPMI 1640 medium. Ultrafiltration (UF) was used for cell removal and HTLV concentration before banding on a sucrose gradient in a continuous flow ultracentrifuge. Cell removal by centrifugation disrupted HTLV particles causing P24 core protein to interfere with concentration of IL-2 using UF and ammonium sulfate recovery methods.

104. Large-Scale Perfusion Cell Culture Systems. J. FEDER*, W. R. Tolbert* and C. Lewis, Jr.*, Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, MO 63167

The growing need to produce large quantities of biologically important molecules such as growth factors, monoclonal antibodies and specific enzymes has focused attention on the development of efficient, large-scale animal cell culture systems. Conventional methods which address most of the nutritional and physical requirements of the cells expose the growing cells to a constantly changing medium environment which contrasts with the near homeostatic condition maintained in vivo. Perfusion systems have been used to achieve a steady-state culture environment with significant advantage over static growth methods. We have developed perfusion cell reactors for both growth of cells in suspensions as well as on microcarriers. These systems consist of suspension vessels containing porcelain filters which are either rapidly rotating or surrounded by rotating agitators for removal of expended medium. Studies with these systems, as well as a perfused flat bed hollow fiber reactor, have shown that both high cell densities and greater cell yield per volume of medium used can be achieved. Such steady-state systems generally require lower serum concentrations and provide greater cell viabilities.

105. Human Tumor Cell Growth in a Prototype Process-Scale Hollow Fiber Culture System. M. C. Wiemann, K. McCarthy, B. Creswick and P. Calabresi, Roger Williams General Hospital, Providence, RI 02908 and J. HOPKINSON, Amicon Corporation, Danvers, MA 01923 (Sponsored by J. M. Quarles*)

A prototype process-scale hollow fiber cell culture system consisting of a culture cartridge, medium reservoir, peristaltic pump and in-line filter in a recirculating loop of silicone tubing was evaluated for its ability to support the growth and maintain the function of large numbers of cells for long periods of time. About 5 x 10^9 cells of the heterogeneous colon carcinoma cell line DLD-1 were inoculated into a culture cartridge containing 1000 hollow fibers with a luminal surface area of 1000 cm^2 and a cutoff of 10,000 daltons. One liter of culture medium supplemented with 10% fetal bovine serum and antibiotics was recirculated at a rate of 100 ml/min; half volumes were replaced twice weekly. Growth was monitored by the rate of glucose consumption, which reached 400 mg/day by the fifth week of culture. Other reliable markers of cell growth were the concentrations of the enzymes LDH and GOT in the cartridge fluid, which rose to levels of 3536 and 848 mU/ml respectively after 40 days. The histopathology of a terminated culture cartridge was that of a moderately differentiated colon carcinoma which resembled the original DLD-1 human tumor. Cells infiltrated the fiber wall matrix and filled the interfiber spaces, growing to tissue-like densities. This culture system allows long term culture of large numbers of cells actively functioning in the production of tumor-specific macromolecules and thus provides significant advantages over other mass culture techniques.

106. Novel Ceramic Material for Large Scale Cell Culture. B. K. LYDERSEN,* G. C. Pugh,* E. C. Duncan,* K. T. Overman, D. M. Johnson, and B. P. Sharma,* Research and Development Division, Corning Glass Works, Corning, NY 14831

Ceramic structures having a relatively high surface area per unit volume (20-60 cm^2/ml) have been found to support the growth of diverse types of animal cells. These include WI-38, MRC-5, BHK, Vero, MDCK, HeLa, RPMI-1788, primary cells from monkey kidney and chick embryo, and cell lines derived from mosquito and trout. Utilizing a system capable of pH and dissolved oxygen detection and control, the culture of MRC-5, MDCK, and Vero on structures having surface area of 4.3 x 10^6 cm^2 resulted in yields of 2.3, 20.7, and 39.9 x 10^3 cells, respectively. Growth rates and uniformity of cell distribution were found to be comparable to that on roller bottles, while maximum cell densities ranged from equal to two-fold greater on the ceramic. Quantitative recovery of cells from the ceramic was routinely obtained and subculturing indicated the viability of these cells was comparable to those harvested from roller bottles. Oxygen consumption rates of the cultures were measured and a correlation to a growth curve was made for one type of cell (MDCK). It was concluded that the measurement of specific oxygen consumption rates can be used to determine the relative stage of the culture.

107. Long-Term, High-Frequency Regeneration from Cereal Tissue Cultures. M. W. NABORS (sponsored by R. Bresan*), Department of Botany and Plant Pathology, Colorado State University, Ft. Collins, CO 80523

Cereal tissue cultures produce at least two types of cells. Regions of small, isodiametric cells (30 μm in diameter)
are referred to as embryogenic callus because they frequently give rise to regenerated plants. Regions of long, tubular cells (50 × 350 μm) are referred to as non-embryogenic callus and do not produce regenerates. Most popular tissue culture media allow preferential growth of non-embryogenic tissue. Thus a typical cereal callus contains mostly non-embryogenic tissue. This accounts for the legendary low frequency plant regeneration in cereals. By visual selection of embryogenic callus in rice, wheat, oats, pearl millet, and proso millet we have been able to obtain over 33 times more regenerated plants per gram of callus when compared to non-selected callus which is mostly non-embryogenic. Regeneration is easily maintained for over one year in all cases. By appropriate manipulation of medium constituents it is possible for each species and cultivar to obtain a medium which substantially increases embryogenic callus production. In many cases a totally embryogenic callus is obtained. (Supported in part by United States Agency for International Development, contract No. AID/DSAN-C-0273.)

108. Salt Tolerance in Cultured Plant Cells. PAUL M. HASEGAWA*, Ray A. Bressan, and Avtar K. Handa. Department of Horticulture, Purdue University, West Lafayette, IN 47907

Cell lines of tobacco (Nicotiana tabacum L. var. W-38) have been obtained which are capable of growing in medium which contains up to 25 g/l NaCl. The mechanism of tolerance to NaCl appears to be at least in part due to adaptation since clones obtained from cell lines grown in medium with 10 g/l NaCl for 15 generations in medium without NaCl. In addition adapted cell lines exhibit decreasing tolerance to NaCl after short periods of growth in medium without NaCl. However, there appears to be some stability of the tolerance after the cells have grown in high salt levels for long periods. The cells adapt physiologically to changes in the external NaCl concentrations by adjusting their internal osmotic potential. This osmotic potential adjustment, however, is not solely for turgor maintenance as turgor increases after adaptation. The osmotic adjustment is partly the result of accumulation of Na+ and Cl− in the cell. Preliminary experiments indicate that Na+ and Cl− are sequestered in the vacuole.

109. The Role of Proline in Bacteria Under Conditions of Osmotic Stress. L. N. CSONKA, Department of Biological Sciences, Purdue University, West Lafayette, IN 47907 (sponsored by R. S. Bressan*)

Exogenous proline specifically stimulates the growth rate of a number of bacterial species under conditions of osmotic stress. We studied this phenomenon extensively with the bacterium Salmonella typhimurium, and found that the uptake of proline into the cells is stimulated by growth in media of elevated osmotic strength. We also isolated proline overproducing mutants of this organism. A number of these mutants proved to have acquired enhanced osmotic stress tolerance, with the intracellular proline levels being correlated with the increases in osmotic stress tolerance. In these mutant strains the intracellular proline levels were regulated such that they were proportional to the osmotic strength of the growth medium. We do not know why increased intracellular proline results in enhanced osmotic stress tolerance; proline may be an inert osmoticum, or possibly it might have more specific interactions with cellular components to permit their functioning in an environment of reduced water activity. We have cloned a number of the mutations that caused proline overproduction and elevated osmotolerance and currently we are attempting to transfer them into other bacterial species in order to test whether high levels of proline would, in general, result in elevated osmotic stress tolerance.

110. Free-radical Resistance and Genetic Segregation in Mutants Derived from Nicotiana tabacum Cell Cultures. K. W. HUGHES*, S. Holekamp and R. W. Holton, Dept. of Botany, Univ. Tennessee, Knoxville, TN 37996-1100

Cell cultures of Nicotiana tabacum were exposed to lethal concentrations of the herbicide Paraquat (methyl viologen). Paraquat forms oxygen-associated free radicals within the cell which in turn destroy cellular membranes and cause cell death. This type of free radical damage has been associated with aging and senescence, with ozone toxicity and with a variety of other biological and chemical agents including other herbicides. Cell lines which were resistant to lethal concentrations of the herbicide were selected. Metabolic analyses on some cell lines indicated that increased levels of free-radical detoxifying enzymes (superoxide dismutase and peroxidase) may be responsible for the resistance trait. Plants were regenerated and were: 1. self pollinated 2. crossed to controls 3. crossed to other mutants. Seeds were germinated. Callus was obtained from seedling tissue and tested for resistance. The resistance trait segregates to the R2 progeny with ratios indicative of Mendelian segregation patterns.

111. Comparison of Serum Dependency of Transformed Insect Cell Lines of Different Degrees of Malignancy. K. ROGER TSANG* and Marion A. Brooks,* Dept. of Entomology, Fish, and Wildlife, Univ. of Minnesota, St. Paul, MN 55108

In vivo malignancy of transformed insect cell lines, cultured in medium with 15% fetal bovine serum (FBS), was expressed as LT50 (in days) following injection of the cells into the hemocoele host insects. In vitro generation times of the cells were compared for 4 malignant lines and a young, untransformed line cultured in 1, 5, 10 and 15% FBS. In 1% FBS the two more malignant lines had generation times (in days) of 6.63 and 5.94 but the two less malignant lines and the normal line failed to grow. At levels of 5, 10 or 15% FBS there was only slight reduction in generation time of the two more malignant lines whereas the two less malignant and the normal line all responded by more rapid growth with the increasing levels of FBS. The data below illustrate a distinctly reduced serum requirement by highly malignant cells compared to less malignant cells.

| Cell line | 1% FBS | 5% FBS | 10% FBS | 15% FBS |
|-----------|--------|--------|---------|---------|
| Generation Time in Vitro | 6.63 | 5.94 | - | - |
| 5 | 4.80 | 4.73 | 8.43 | 8.43 | 12.73 |
| 10 | 4.48 | 4.22 | 6.20 | 5.02 | 7.77 |
| 15 | 4.40 | 3.94 | 5.02 | 3.73 | 3.08 |
| LT50 in Vivo | - | - | 63 | 71 |

(Supported in part by NSF Grant No. PCM-8012013)
112. Fatty Acids of Four Cell Lines of One Species of Insect and Lines Adapted to Serum-free Medium. GORDON B. WARD and Marion A. Brooks,* Dept. of Entomology, F. & W. L., University of Minnesota, St. Paul, MN 55108

The fatty acids from three lipid fractions of insect cell cultures grown in a medium containing 10% fetal bovine serum (FBS) were identified with gas-liquid chromatography. Although the four cell lines were derived from the same species (Blattella germanica), they are morphologically distinct and contain different arrays of fatty acids. One of the greatest differences is the high percentage of oleic acid in the phospholipid fraction of line UMN-BGE-59 — approximately twice that of the other lines. The cells of 59 attach more securely to the flask than the other cells do. There were no other outstanding variations in single fatty acid levels among the three remaining lines. Two of the lines, UMN-BGE-1 and UMN-BGE-2, were adapted to grow in serum-free medium containing an emulsion of cholesterol and linoleic acid. Under these conditions, the phospholipid fraction of the cells of line 1 contained seven times more linoleic acid than they did when grown in FBS medium.

113. Comparative Culture of Insect Cells from the Lepidoptera, Coleoptera, and Orthoptera in Various Media. R. H. GOODWIN,* Rangeland Insect Laboratory, USDA/ARS, Montana State University, Bozeman, MT 59717

Cell lines from three orders of insects were cultured in media designed for Lepidopteran cells, Orthopteran cells, and Dipteran cells. The salt ratios of the various media were readjusted to allow cross-culturing of the cell lines and some primary cultures in media not originally designed for cells from the given taxonomic group. Various supplementing sera were used at low concentrations, and the cells were also passaged in serum-free media variations. Cell responses were monitored by survival times and growth rates in the different media. Estimated and tested media differences responsible for the observed performance of the three insect groups will be described. Media tested were variants of Goodwin’s IPL-52B medium, Mark’s M20 medium, and Schneider’s Drosophila medium.

114. Development of a Cell Line from the Coleopteran Insect, Diabrotica undecimpunctata. D. E. LYNN,* USDA, ARS, Insect Pathology Laboratory, Beltsville, MD 20705

The Coleoptera (beetles) are the largest order of insects and include a number of agriculturally important pest species. It is of interest to use cell cultures of these insects in studies of insect pathogens. Unfortunately, cell cultures of Coleoptera are nearly non-existent, with only one cell line recently reported. We initiated cultures from several tissues of the southern corn rootworm, Diabrotica undecimpunctata, including minced tissues of several embryonic stages, minced newly hatched larvae, larval hemocytes, fat body and cuticular epidermis. Several media were utilized (including IPL-41, IPL-52B, and IPL-76) with concentrations of fetal bovine serum (FBS) from 8 to 20%. The most successful cultures were derived from embryos in early organogene-sis minced in IPL-52B medium with 8% FBS. A cell line has resulted which consists of both epithelial-like and spindle-shaped cells. Approximately 65% of the cells are diploid (2n=20) and growth rate is currently at a 6 day doubling time. Cells are currently growth in a combination of 3 parts IPL-52B and 1 part IPL-76 with 8% FBS. Cells are subcultured by trypsinization and have been passaged 10 times.

115. Expression of Gamma-Glutamyl Transpeptidase Activity in Primary Cultures of Hepatocytes Cocultivated with a Hepatocellular Carcinoma. L. B. JACOBSEN* and S. J. Beecher, Medicinal Chemistry and Pharmacognosy, School of Pharmacy, Purdue University, West Lafayette, IN 47907

Gamma-glutamyl transpeptidase (GGT) activity is used as an in vivo marker of hepatocarcinogenesis in the rat. Normal liver cells are negative; foci of GGT positive cells develop in livers of rats treated with hepatocarcinogens. Primary cultures of hepatocytes tend to retain GGT activity corresponding to that expressed in vivo. However, primary cultures of hepatocytes from normal liver express GGT activity after several days in culture if they are maintained on collagen gel/nylon meshes (Sirica et al., PNAS 76: 283). Primary hepatocyte cultures plated in plastic dishes do not express GGT activity. A line of hepatocellular carcinoma cells (PCCL-H) derived from a carcinogen treated rat is strongly GGT positive. In cocultures of these cells (10-50 hepatocytes: 1 PCCL-H cell), many hepatocytes in close association with PCCL-H cells express intense GGT activity within 24 hours after plating. Cultures contain hepatocytes alone, hepatocytes incubated with conditioned medium from PCCL-H, cultures, or hepatocytes cocultured with WI-38, HeLa, or KB cells did not express GGT activity even after 72 hours.

(Supported in part by NCI Grant CA 33441)

116. A Gelatin Microcarrier for Cell Culture. M. S. PARIS,* D. L. Eaton, D. E. Sempolinski and B. P. Sharma,* Research and Development Division, Corning Glass Works, Corning, NY 14831

A variety of microcarriers are currently available for the support of attachment dependent cells in suspension culture. The two most prominent difficulties facing each is the removal of cells from the surface and/or separation of eluted cells from these spent microcarriers. We have developed a gelatin derived microcarrier which supports the growth of human lung and foreskin fibroblasts as well as epithelial cell lines such as MDCK, Vero, and swine testicle. Attachment and spreading over the surface of the gelatin has been observed to be completed within 4-5 hours for several cells. Spinning cultures resulted in especially high yields of swine testicle cells on the gelatin microcarrier where the 5-day yield (cells harvested/cells seeded) ranged from 7-11. In comparison to the Cytodex 3, a collagen coated microcarrier, yields on the gelatin microcarrier were 2-3 times as great. This microcarrier, developed under a license, dissolves completely in about 12 minutes using a 0.13-0.25% trypsin or a 0.4% dispase solution. The procedure does not adversely affect cell viability upon harvesting.
117. Impedance Measurements of Mammalian Cells in Tissue Culture. I. GIAEVER and C. R. Keese,* General Electric Research and Development Center, P.O. Box 8, Schenectady, NY 12301

Both normal (WI-38) and transformed (WI-38 VA13) fibroblasts have been cultured on evaporated gold electrodes subjected to an alternating electric field at 4000 Hz. The electrodes and the tissue culture medium are good conductors, and the measured impedance is due mainly to the boundary between the electrodes and the culture medium. The applied electric field produces a voltage drop at the boundary of a few millivolts at a current density of a few milliamps/cm² and has no noticeable effect on the cells. The cells, however, have a marked effect on the measured impedance and cause it to fluctuate with time. The amplitude of the fluctuations is greatly reduced in the presence of cytochalasin B (5 μg/ml) or demecolcine (0.8 μg/ml) suggesting it results from cell movement on the electrodes. By making the electrodes very small (10⁻⁴ cm²), one can observe changes and fluctuations in impedance due to a single cell.

(Supported, in part, by a grant from the National Foundation for Cancer Research.)

118. Comparison of ²¹²Pindolol and ³H-Dihydroalprenolol Binding to Intact Myocytes in Culture. L. TERRACIO*, C. G. Ingebretsen and W. R. Ingebretsen, Departments of Anatomy and Pharmacology, University of South Carolina, Columbia, SC 29208

Characterization of the beta-adrenergic receptor populations on myocardial cells in vitro would be a useful tool for cardiac cell biologists. However, work in this area has been hampered by the lack of a ligand compatible with intact myocytes. In this study we have compared the binding of ³H-dihydroalprenolol (³H-DHA — a commonly used beta-adrenergic receptor agonist) and ²¹²Pindolol (a new beta-adrenergic agonist) to intact neonatal and adult myocytes in culture. Neonatal myocytes were isolated from 2-5 day old rat ventricles using a collagenase digestion and selective attachment procedure. Adult ventricular myocytes were isolated from 250-400 gm rats by perfusion with collagenase and hyaluronidase, followed by serial digestion in 0.25% trypsin and a selective attachment procedure. One to three week old cultures were washed with serum free media (2X) and incubated with either ³H-DHA or ²¹²Pindolol under conditions to maximize specific binding of each ligand. The stereospecificity of the binding of both ligands was investigated in competition studies using the stereoisomers of propranolol (agonist) and isoprotenerol (agonist). ³H-DHA bound to intact myocytes in culture but the binding was not saturable and was displaced non-stereospecifically by beta-adrenergic agonists and antagonists. ²¹²Pindolol bound to intact myocytes with high affinity and was displaced by beta adrenergic agonists and antagonists in a stereospecific manner. ²¹²Pindolol appears to be superior to ³H-DHA as a probe for beta-adrenergic receptors in intact myocytes. (Supported by Biomedical Research Support Grant #0657RR05815B, The American Heart Association and the South Carolina Heart Association.)

119. Isolation, Culture and Characterization of Adult Human Hepatocytes from Surgical Liver Biopsies. F. BALLET*, M. E. Bouma, S. Wang, N. Amit, R. Infante, INSERM U.9, Hôpital Saint-Antoine, F 75012 PARIS - FRANCE

A technique for the isolation and culture of human hepatocytes from biopsy fragments has been developed, based on those described by Reese (In Vitro 17: 935, 1981) and Strom (J. Natl. Cancer Inst. 68: 771, 1982). The conditions of collagenase (Col.) perfusion through the main portal vein of the biopsy and further cell dissociation and culture were first optimized using pig liver fragments. Human biopsy fragments (mean = 10 g), obtained during hepatic resections, were perfused with a Col. solution; after dissociation the yield range was 1 to 4 x 10⁶ cells/g liver. Hepatocyte yield depended on (a) the Col. batch and (b) the anatomical disposition of portal triads in the sample. Viability averaged 85% and was influenced by the hypoxia period between vascular clamping and sectioning of the biopsy. Hepatocytes were immediately plated on uncoated plastic dishes and maintained in primary monolayer culture in a conventional medium for 24 h and then in a serum-free medium containing hydrocortisone for about 8 days. Cell morphology and histochemical characteristics were identical to hepatocytes in vivo. Typical bile canaliculi were observed by electron microscopy. Fine lipid Oil Red + droplets appeared early in the cytoplasm and progressively increased in spite of significant lipoprotein secretion into the medium. Albumin secretion rate was maximal (34 μg/24 h/10⁶ cells) 5 days after plating. We are presently using this model to study plasma lipoprotein metabolism and xenobiotic transformation in adult human hepatocytes.

120. The Efficacy of Incyte™ as a Surface Disinfec-
tant for Plant Tissue Culture explants. S. E. HYNDMAN, Oglesby Nursery, Inc., 3714 SW 52 Ave., Hollywood, FL 33023 (Sponsored by R. P. Oglesby*)

Incyte™ (a chlorine dioxide complex) is known to be an effective bacterial, fungal, and viral sterilant. In this study, Incyte™ and sodium hypochlorite were compared for use on Boston fern rhizome tip explants to examine the surface disinfection qualities of these compounds. Sixty-five percent of the cultures became infected with microbial contaminants when the explants were disinfected with 0.53% sodium hypochlorite for 10 min. Of those cultures which were contaminated, 8% were infected with fungi and 92% were infected with bacteria. Incyte™ appeared to be a more effective bactericide than sodium hypochlorite as only a very few of the cultures inoculated with explants treated with this compound contained bacterial contaminants. However, a higher frequency of the cultures inoculated with explants disinfected with Incyte™ contained fungal contaminants as compared to those cultures whose explants were surface disinfected with sodium hypochlorite. These results suggest that a surface disinfection procedure using Incyte™ and sodium hypochlorite may be very effective against microbial contaminants. However, for explants that have been prepared for disinfection by removal of the outer layers of tissue that might harbor fungal spores, Incyte™ alone may be the most appropriate disinfectant. (Supported by the Alcide Company, Farmingdale, New York)
122. Rooting Apple Cultivars In Vitro: Interactions of NaCl on Soybean Cells in Suspension

Cuttings dark treated for 1 week further increased rooting up to 60%. Adding PG to the rooting medium of 'Delicious' apple, its spur-type strains, and some other cultures from 2 different sources responded similarly to the rate of root development but appeared to have no effect when PG was absent. Moving the cuttings to auxin-free medium has only a moderate or no effect on these cultures (suspension culture). The observed effects of NaCl may be either due to a change in osmotic potential or due to an ion effect. (Supported by USDA/CR Grant No. 7903-1-PS2)

123. Effect of NaCl on Soybean Cells in Suspension Culture and on the Regenerative Ability of Intercotyledonary Nodes. T. M. CURRY, S. M. Bhatti and P. S. Kahlon,* Biology Department, Tennessee State University, Nashville, TN 37203

Two Soybean (Glycine max L. Merr.) lines (SB4 and SB3) were maintained in Gamborg’s B5 medium. Cells from these cultures were exposed to different concentrations of NaCl added to the medium. Growth was measured by pack cell volume, fresh weight and dry weight of cells. Results show that addition of NaCl at low levels (0.1% and 0.25%) had a stimulatory effect as measured by an increase in growth. Approximately 50% reduction in growth was observed when 0.5% NaCl was added to the medium. Cells in 1.0% NaCl plasmolyzed and died within a day. In a separate experiment the effect of NaCl on plantlets was investigated. Intercotyledonary nodes from cultivars Miles, Centennial, Tracy and Williams were used. Seedlings were conditioned on Murashige-Skoog (MS) medium supplemented with 5 mM benzyladenine. Nodes were then placed on MS media with different concentrations of NaCl. The frequency of bud formation was recorded. Addition of low concentrations (0.1% and 0.25%) of NaCl increased the frequency of shoot bud formation. As observed in suspension culture, the addition of higher levels of NaCl (0.5% and 1.0%) reduced or inhibited shoot bud formation. The observed effects of NaCl may be either due to a change in osmotic potential or due to an ion effect. (Supported by USDA/CR Grant No. 7903-1-PS2)

124. Ionic Currents Along the Surface of Carrot Embryoids Measured with the Vibrating Probe. C. E. BARR and D. K. Dougall,* W. Alton Jones Cell Science Center, Old Barn Road, Lake Placid, NY 12946

The vibrating probe developed by Nuccitelli and Jaffe was used to measure the ionic currents at various loci along the surface of submersed embryoids of the garden carrot, Daucus carota. Each embryoid was several mm long and had a well formed primary root; the two presumptive cotyledons were poorly organized, somewhat callus-like in appearance. In general there was a complex mosaic of local currents over the entire surface of the embryoid. Some semblance of pattern was discernible along the root surface but not along the presumptive cotyledons. This suggests that organized patterns of current flow are associated with organized growth. The current densities were on the order of 1 μA cm⁻² at a distance of about 40 μm from the surface, both for roots and presumptive cotyledons. The positive current travelling out of the tissue is presumably carried by H⁺. The inward current was most probably a calcium ion current in this case since the medium consisted of 0.5 mM calcium chloride. Under normal conditions the dominant inward current pattern along the root surface was very similar to that for normal seedlings.

125. Promotion of Bulbing in Narcissus (Daffodil) Shoots in Vitro. J. E. A. SEABROOK* and B. G. Cumming, Research Station, Agriculture Canada, P.O. Box 20280, Fredericton, N.B., E3B 4Z7 and Biology Department, University of New Brunswick, Fredericton, N.B., E3B 6E1, Canada

Narcissus (Daffodil) plantlets produced in vitro generally have poor survival when transferred to the greenhouse. Transplant shock can be reduced by inducing the small shoots to produce rooted bulbs in vitro. Bulbing in vitro was induced by the inclusion of manitol (8 g/L) in the medium, and by substituting glucose (16 g/L) for sucrose as the carbon source. The addition of increased KH₂PO₄ and KHCO₃, to modified Knudson’s macro-nutrients and Heller’s micronutrients also promoted bulbing. Activated charcoal (0.1%) and 5.4 μM (1 mg/L) NAA improved bulbing and promoted rooting. Bulbed plantlets produced in vitro were frequently...
dormant. Dormancy was broken by a cold treatment of 10 weeks at 8°C or by the application of 0.1 μM (0.035 mg/L) GA for 24 hours.

126. Screening Alfalfas Adapted to the Southwestern United States for Regenera
type G. C. PHILLIPS,* Department of Horti-
culture, New Mexico State University, Las Cruces,
NM 88003-3530
To apply cell selection procedures in plant improvement, it is desirable to use materials generally adapted to the region of intended use to speed up cultivar development. The only regenerator populations of alfalfa (Medicago sativa L.) previously identified were derived from the northcentral U.S. region (Bingham et al., Crop Sci. 15: 719–721, 1975). Over 300 genotypes of alfalfa adapted to the southwestern U.S. region derived from 3 distinct genetic populations were screened for regenerator lines with about 11% responding positively. No significant difference for regenerator frequency among populations was detected. The optimal regeneration scheme involved callus induction on Schenk and Hildebrandt medium (Can. J. Bot. 50: 199–204, 1972) containing 0.06 mg/l picloram (4-amino-3,6,6-trichloropicolinic acid) and 0.1 mg/l 6-benzylaminopurine (Phillips and Collins, Crop Sci. 19: 59–64, 1979) and transfer to medium containing 0.01 mg/l 2,4-dichlorophenoxyacetic acid and 2.0 mg/l adenine (Phillips and Collins, Crop Sci. 20: 323-326, 1980) for 1 or 2 passages to induce somatic embryogenesis, following the regeneration scheme previously developed for red clover (Trifolium pratense L.). Some genotypes responded only to the red clover regeneration scheme, some responded only to the standard alfalfa regeneration scheme, and some responded to both, suggesting the possibility that genotype by regeneration scheme specificities may exist.

127. Morphogenetic Responses of Cultured Hypocotyl and Cotyledonal Segments of Leucaena. R. Nagami and S. VENKETESWARAN,* Department of Biology, University of Houston, Houston, TX 77004
Hypocotyl and cotyledonal segments from aseptically germinated seedlings of two varieties of Leucaena leucocephala (Lam.) de Wit. were cultured on a simple mineral salt-sucrose basal medium (BM). After 2 weeks, the explants produced callus or leafy shoots from their cut ends. Addition of 2,4-dichlorophenoxyacetic acid (2,4-D) or α-naphthalene acetic acid (NAA) at 0.5, 1.0, 2.0, 5.0 and 10.0 mg/l to basal medium enhanced callus formation compared to the BM alone. When kinetin (0.5, 1.0, 2.0, 5.0 and 10.0 mg/l) was added to BM + 2,4-D (0.5 mg/l), there was no difference in callus proliferation; but a combination of NAA (0.5 mg/l) and 6-
Benzylaminopurine (BAP) at 0.5, 1.0, 2.0, 5.0 and 10.0 mg/l induced increased proliferation. Transfer of explants with the callus from NAA and BAP (all concentrations) media to BAP (2.0 mg/l) medium resulted in regeneration of leafy shoots from both the callus and the remnants of the explant. Explants of L. diversifolia (Schlecht) Benth. produced callus and somatic embryos on BM + 2,4-D (0.5 mg/l). These somatic embryos upon transfer to BM + BAP (2.0 mg/l) differ-

128. Regeneration and Pigment Formation in Tissue Cultures of Leafy Spurge (Euphorbiaceae). David G. DAVIS, Sponsored by Edwin P. Marks*, USDA, SE, ARS, Metabolism and Radiation Research Laboratory, State University Station, Fargo, ND 58105
Plants have been obtained from callus, cell suspension and isolated root cultures of leafy spurge, genus Euphorbia. The species designation is indefinite at this time. Several variants were designated as biotypes, based on persistent leaf characters. Eight biotypes were grown in liquid B5 medium with 1 mg/L 2,4-dichlorophenoxyacetic acid. Striking variations in cellular morphology of four biotypes range from nearly single spherical cells, to elongated cells attached end to end, to extremely large clumps. The clumpy biotypes formed roots readily in liquid medium without growth regulators. The single cell cultures formed a few root initials in the few cell clumps that formed. Tracheary element formation varied with each biotype. One biotype formed tracheids and proembryo masses readily, and produced light-induced red pigments (presumably anthocyanins) on a medium designed for the regeneration of tobacco protoplasts. This contrasts to other biotypes in which no red pigments were observed under similar conditions. Pigmented cells grew on the surface of the callus, so oxygen may be a factor in pigment formation. Plant initiation occurred in a few instances under a variety of conditions: in liquid cultures in the dark without growth regulators, and in callus on agar with 10 μM abscisic acid followed by transfer to light on medium with or without 4.6 μM kinetin.

129. Thiadiazuron Effects on Soybean Callus and Radish Cytokinin Bioassays. J. C. Thomas* and F. R. H. Katterman, University of Arizona, Tucson, AZ 85721
Cytokinin dependent callus of Glycine max cv. “Acme” exhibits vigorous growth in the presence of thiadiazuron (N-phenyl-N’-1,2,3-thiadiazol-5-ylurea). This auxin type cytokinin allows callus fresh weight to increase when present in the culture medium at less than 0.5 μg/L. Maximum growth occurs at 1.0 mg/L; at higher concentrations growth was suppressed. Thidiazuron is also very active in the radish cotyledon assay for cytokinins. With this assay the maximum response approximates 5 × 10^−6 M 6-benzyladenine when thidiazuron is present at 0.1 mg/L. At the higher concentrations tested, suppression of cotyledon expansion was not significant. Mok [Proc. Nat. Acad. Sci 76: 3880–3884 (1979)] has suggested some auxin type cytokinins may stimulate endogenous cytokinin synthesis in normally cytokinin dependent tissues. Thus cytokinin metabolites of thidiazuron and kinetin grown soybean callus were examined. These results (with regard to endogenous cytokinin synthesis) will be discussed.
130. Some Characteristics of Cell Suspensions of Sweet Potato (Ipomoea batatas) and Tomato (Solanum lycopersicoides). J. H. M. Henderson, Senior Research Professor of Biology, Carver Research Laboratories, Tuskegee Institute, Tuskegee, AL 36088

The sweet potato (Ipomoea batatas) and a wild relative of the common tomato, Lycopersicon Sp., Solanum lycopersicoides, are desirable plants from which to make cell suspensions because of the economic importance of these two plants. Three cultivars (CV) of I. batatas have been used for this work (‘Carver’, ‘Centennial’ and ‘Jewel’) and a single species of S. lycopersicoides (SL). The basal medium used was Murashige-Skoog (MS) with modifications. Several methods were used to initiate CS. These include agitation on a gyratory shaker. Two methods of assessing growth have been used: measurement of the packed density of cell aggregates and small calli and measurement of turbidity (nephelometry). CS varies in size from very integral, single cells to large aggregates. Both light and scanning microscopy show the structure and morphology of these entities in fine detail. By altering the auxin: cytokinin composition, some degree of differentiation has occurred. The growth of ‘Jewel’ CS shows a slow rate for the first 6-7 days, then a rapid rate for the next 6-7 days and a slowing down for the final 7-8 days, after which necrosis begins. ‘Jewel’ CS apparently has formed a fast-growing mutant strain which when placed on agar medium produces a callus which is different in both appearance and growth rate from the original clone.

131. In Vitro Assay to Detect Chemical Inhibitors of Gap Junction-Mediated Intercellular Communication. C. JONE, J. E. Trosko*, L. Parker and C. C. Chang, Dept. of Pediatrics/Human Development, Michigan State University, East Lansing, MI 48824

Intercellular communication within and between cells of various tissues is a fundamental biological process required to orchestrate complex mechanisms regulating cell proliferation and differentiation. Inhibition of intercellular communication (caused by cell death, cell removal, endogenous or exogenous chemicals) can trigger either adaptive or nonadaptive responses of cells, depending on circumstances. Chemical inhibition of intercellular communication has been speculated to play roles in the mechanisms of teratogenesis, tumor promotion and reproductive dysfunction. We have tested a wide variety of non-mutagenic chemicals at non-cytotoxic doses for their ability to inhibit metabolic cooperation in a Chinese hamster V79 cell system. Several known tumor promoters of various organ systems, teratogens and reproductive toxicants were shown to block metabolic cooperation. With known receptor-dependent tumor promoters (i.e., TPA and telocidin), a ‘down-regulation’ of inhibition of metabolic cooperation was observed. A disappearance of gap junctions has been observed during chemical inhibition of metabolic cooperation. Chemicals having the ability to interfere with gap junction-mediated intercellular communication have the potential of being teratogens, tumor promoters and reproductive toxicants.

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132. Modifications of Gene Expression and Differentiation in Rat Tracheal Epithelial Cells Exposed to a Tumor Promoter. V. E. STEELE* and R. Wu*, Northrop Servies, Inc., Research Triangle Park, NC 27709 and W. Alton Jones Cell Science Center, Lake Placid, NY 12946

Since TPA (12-0-tetradecanoylphorbol-13-acetate) has been shown to enhance carcinogenesis both in vivo and in vitro with rat tracheal epithelium, the same system was used to study alterations in gene expression and cellular differentiation following TPA exposure. Epithelial cells were isolated from Fischer F-344 male rat tracheas. The cells were plated onto collagen-coated dishes in medium containing 0, 1, 10, or 100 ng TPA/ml. At seven days a portion of the cultures were dissociated to determine cell number and a portion labeled with "C-amino acids to determine patterns of protein synthesis. To begin determining TPA-induced changes in cellular differentiation, keratin was extracted from the labeled cells and analyzed by polyacrylamide gel electrophoresis. An increase in cell number per culture was observed in cultures exposed to TPA. The 10 ng TPA/ml group showed a 22 fold increase as compared to controls. Gel electrophoresis studies using keratin protein extracts showed over a 3 fold increase in total keratin being synthesized and, in particular, increased synthesis in the 48-59K region. Quantitative analysis of the autoradiograms revealed several bands which showed dose dependent increases in new synthesis. The results of these studies indicate that TPA stimulates cell proliferation and alters gene expression in cultured tracheal epithelial cells.

133. Balb-3T3 Cell Response to Extracts of Organic Air Samples as Seen by Their Survival in Aggregate Form. J. T. ZELIKOFF, N. Atkins, E. V. Orsi* and T. J. Kneip, New York University Medical Center, Inst. of Env. Med., 550 1st Avenue, NY, NY 10016 and Seton Hall University, South Orange, NJ 07079

Extracts of organic air samples have been shown to possess components capable of transforming cells. Using anchorage independence as the main criterion, transformation was demonstrated by the formation of cell aggregates in semi-solid medium, a method having the ability to produce colonies in a significantly shorter time than soft agar technique. This study was done to determine if cells exposed to extracts of air samples could, unlike their normal counterparts, divide in agar to form aggregates; and would these cells demonstrate a dose response phenomena. A clone of 3T3 cells was exposed to increasing concentrations of extracted air samples, and to benzo(a)pyrene which served as positive control. Air samples were a composite from one city, but consisted of either non-polar(I) or polar(II) material. Cells were exposed to test compound for 72 hours and monitored for aggregate formation over 6 days. Untreated and solvent treated control cells failed to form large aggregates and showed a decline in cell number following seeding at day 0. Cells treated with organic fractions I or II showed a dose response increase in cell number along with the formation of progressively larger aggregates, findings similar to those seen with the positive control. Results agree with those demonstrated in the simultaneously run
colony assay. The findings show that this rapid assay is capable of detecting the transforming activity in environmental samples. (Supported in part by Grants from NIH and the American Petroleum Institute EHA.)

134. Limited Attachment-Independent Growth in a Neurofibromatosis Skin Fibroblast Line. J. J. FREED* and Mary Ellen Croke, The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111

In experiments to test whether certain human genes predisposing to cancer alter the susceptibility to transformation of skin fibroblasts, we have observed an elevated frequency of attachment-independent colonies in a cell line from the unaffected skin of a patient with neurofibromatosis (von Recklinghausen disease, NF). Cells from normal donors or those with dominant genes predisposing to cancer, either exposed to nitrosoguanidine (MNNG) or maintained as untreated controls, were propagated for 20 population doublings in monolayer. At each 1:10 subculture, cells were plated in soft agar to assay for attachment-independent growth. Normal cells gave colonies at a frequency near \(1 \times 10^{-4}\), increased less than 10-fold by MNNG. Hereditary retinoblastoma cells were indistinguishable from normals; Gardner syndrome cells plated at \(4 \times 10^{-4}\), increased to \(1 \times 10^{-3}\) by MNNG. NF cells gave higher frequencies of larger colonies: \(6 \times 10^{-4}\) untreated and \(1 \times 10^{-4}\) following MNNG. The NF colonies, although composed of apparently viable cells, showed limited proliferation in monolayer culture. This behavior of the NF cell line might stem from the presence of a minority cell type differing from normal dermal fibroblasts. NF patients (as did the donor of our cell line) may have benign cutaneous neurofibromas: he attachment-independent cells we observe may indicate the presence in the original biopsy of cells that behave as if "partially transformed" in a cell culture assay.

135. X-Ray-induced Chromatid Damage in Cells from Cancer-prone Individuals Implicates DNA Repair Deficiencies. K. K. SANFORD*, R. Parshad, and G. M. Jones, National Cancer Institute, Bethesda, MD 20205 and Howard University, Washington, DC 20059

Ten lines of skin fibroblasts from individuals with genetic disorders predisposing to a high risk of cancer were compared with 9 lines from normal donors with respect to chromatid damage following X-irradiation during G2 phase. The 10 cell lines were from 5 genetic disorders, Bloom's syndrome (BS), familial polyposis (FP), Fanconi's anemia (FA), Gardner's syndrome (GS), and xeroderma pigmentosum, complementation groups A (XP-A), C (XP-C), E (XP-E) and variant (XP-Va). The incidence of chromatid breaks in 5 of the mutant lines BS, FP, FA, XP-C and XP-Va, was significantly higher than in the normal lines. The incidence of chromatid gaps in all mutant lines except XP-A and XP-Va was significantly higher than in the normal lines. Since each chromatid apparently contains a single continuous DNA double strand, chromatid breaks and gaps represent unrepair DNA strand breaks arising directly or indirectly during excision repair of X-ray-induced DNA damage. These cytogenetic data together with results from use of the DNA repair inhibitor, \(\beta\)-cytosine arabinoside, implicate a deficiency in DNA repair mechanisms operative during G2-prophase period of the cell cycle in all of these mutant cell lines. No such deficiencies occur in any of the lines from normal individuals. These DNA repair deficiencies are apparently associated with a genetic predisposition to a high risk of cancer.

136. Analysis of Intra- and Inter-Experimental Variation and Trend in a Standardized Assay for Chemical Enhancement of Viral Transformation in Hamster Embryo Cells. G. G. HATCH*, T. M. Anderson, Environmental Toxicology Division, Nortrop Services, Inc., Res. Tri. Park, NC 27709

Enhancement of DNA (SA7) viral transformation of hamster embryo cells by chemical agents has been successfully employed for over ten years to assay for potential carcinogens. Efficient use of this bioassay requires standardization of test reagents and protocols and determination of experimental variation and trend. Three compounds known to produce positive (benz(a)pyrene and N-methyl-N':nitro-N-nitrosoguanidine) and negative (anthracene) responses in this system were selected to generate results for quantitative analysis. To study variance within each test, the mean numbers of colonies and foci \(\pm\) the standard deviation (SD) at each chemical dose were determined. To examine the trend or consistency within individual experiments, the fraction of cells surviving chemical treatment, and the corresponding transformation frequencies were expressed as a function of log chemical dose and analyzed by linear regression. The reproducibility of replicate experiments was tested by determining the SD's of toxicity data and normalized transformation frequencies. Analysis of individual plate counts and correlation coefficients demonstrated a high degree of internal consistency. The degree of positive response to the test agents related to the slope of the regression line for transformation frequency. These methods permit a quantitative definition of the response of the SHE-SA7 system which improves its utility in screening test agents for transforming potential. Supported by NIEHS/NTP Contract #N01-ES-15796.

137. Viral Transformation Influences Repair of DNA-protein Crosslinks in Excision Deficient Human Fibroblasts. W. G. TAYLOR*, R. Gant*, R. F. Camalier and E. V. Stephens, National Cancer Institute, Bethesda, MD 20205

Xeroderma pigmentosum (XP) is an inherited dermatosis characterized in part, by deficient DNA excision repair of UV-induced dimers and by a frequent predisposition to cancer. When compared to foreskin fibroblasts, XP cells appear more sensitive to low intensity visible light-induced injury and fail to repair competently DNA-protein crosslinks induced with trans-dichlorodiaminoplatinum(II) (DDP). To test whether repair of DDP-induced DNA-protein cross links is associated with cell division in XP cells, disappearance of crosslinks was assessed in slowly dividing XP 20S cells (Group A) and its rapidly growing stable SV40-transformant by the membrane alkaline elution procedure of Kohn. Prelabeled cells were treated with 20 \(\mu\)M DDP, and treatment medium replaced 2 hrs. later with DME
138. Kinetics of Histone Acetylation and Deacetylation in Human Diploid Fibroblasts. M. R. DUNCAN*, M. J. Robinson, and R. T. Dell’Orco*, The Samuel Roberts Noble Foundation, Inc., Box 2180, Ardmore, OK 73402

It was recently reported that hepatoma tissue culture cells contained a small, distinct population of histone H4 which was very rapidly hyperacetylated compared to the majority of H4 molecules. In our studies we first determined whether human diploid fibroblasts (HDF) contained populations of histone H4 differing in their rates of hyperacetylation. Cells were pulse-labeled with [H]-acetate and then chased in the presence of sodium butyrate. At times following initiation of the butyrate chase histones were extracted and separated by electrophoresis. A fluorogram of the gel allowed quantitation of radioactivity in each H4 subfraction, and thus a determination of rates of hyperacetylation. Two distinct rates of H4 hyperacetylation were determined. The majority of H4 molecules were slowly hyperacetylated with a t½ = 140-200 minutes for monoacetylated H4 while a small fraction was rapidly acetylated (t½ = 10-15 minutes for monoacetylated H4). Using pulse-chase experiments in the absence of butyrate we also determined that all core histones were deacetylated with 2 distinct rates (for H4 the rapid rate had t½ = 5 minutes and the slower rate t½ = 30-45 minutes). We also determined that senescent populations of HDF contain both rapidly and slowly hyperacetylated populations of core histones. Possible implications of the presence of a rapidly-hypermodified population of core histones will be discussed.

139. Platelet Derived Growth Factor: Purification and Characterization. T. DEUEL

No abstract received

140. Chemistry and Function of Somatomedin/Insulin-Like Growth Factors and Their Receptors. J. F. PERDUE (Sponsored by T. Maciag*), Lady Davis Institute for Medical Research, Montreal, Canada H3T 1E2

Studies of growth hormone (GH)-dependent skeletal growth have correlated its magnitude with the plasma levels of factors termed somatomedin or insulin-like growth factors (IGF). Subsequent purification has resolved them into two classes, e.g. Sm c/IGF-I and IGF-II, with similar Mr, i.e. 7,500, and biological properties but with different amino acid sequences and pI. Although both IGF-I and -II are presumably involved in skeletal growth, the latter appears to be more important than IGF-I in fetal development. The binding of [125I]-IGF-I or -II to membranes isolated from different tissues, e.g. liver and adipocyte and different species was not equal and indicated that two receptor forms existed.
144. Genetic Characterization of Mutants from Cell Culture: Cosegregation of Altered Enzymatic Phenotype with Selected Culture Trait in Progeny of Regenerated Plants. M. K. B. ERNST, Herbet H. Lehman College/City University of N.Y. and N.Y. Botanical Garden, Bronx, NY 11045

Plant cell systems in which cell culture variants have been regenerated and the variant trait established as a mutation by testing progeny of the regenerated plants will be reviewed. The isonicotinic acid (INH)-resistant mutants of Nicotiana tabacum serve to demonstrate the cosegregation of a biochemical phenotype (inhibition of glycine decarboxylation by INH in crude mitochondrial extracts) and the selected growth trait in culture (ability to grow in the presence of INH in the culture medium) of progeny of crosses and selfings of the regenerated plants and their seed progeny. Genetic segregation data indicate that for Inh 24, resistance is dominant and both dominant and recessive alleles are present in plants regenerated from originally haploid cultures. Heteroploidy, revealed by microspectrophotometric analysis, was not correlated with the INH-resistant phenotype. The importance and difficulties of demonstrating the culture-selected trait in the regenerated plant will be discussed and illustrated by consideration of glycine hydroxamate-resistant and "oxygen-resistant" variants of tobacco.

145. Heritable Somaclonal Variation in Wheat. P. J. LARKIN, S. A. Ryan, R. I. S. Brettell and W. R. Scowcroft, CSIRO Division of Plant Industry, Box 1600, Canberra, Australia 2601

Tissue cultures were initiated from immature embryo segment of a number of wheat genotypes, notably the line Yaqii 50E. After various periods on callus maintaining media from 2-8 months, plants were regenerated. As many as 150 plants could be obtained from the culture of one embryo. Regenerants were analyzed for various morphological, quantitative and biochemical traits in two subsequent selfing generations. The initial regenerant is termed SC1, the following selfed generations are SC2, SC3, etc. Individuals from about 150 of the 525 Yaqii 50E-derived SC families (including all of the most variable families) were examined cytologically. All were normal 2n = 42 hexaploids with no gross chromosomal aberration. The gliadin proteins when separated on gradient polyacrylamide showed the main parental features. This serves as an internal control against pollen contamination or stray seed contamination. But some somaclones had specific gliadin changes which proved to be simply inherited. Simple segregation was also observed for variants with white seeds (parent red); fully awned variants (parent tip awned); awnless variants; plant height increased (parent is rht 1, rht 2); plant height decreased; dark glume colour (parent white glumed); reduced leaf wax. Variants were found with heritable changes in heading date, some much earlier and some much later. Some variants also displayed a heritable reduction in tiller number.

146. Chromosomal and Mendelian Variability in Cultures and Regenerated Plants. T. J. ORTON (Sponsored by R. H. Lawrence*), Applied Genetics Lab, Agrigenetics Corporation, 3375 Mitchell Lane, Boulder, CO 80301

Experiments were conducted in which multiply heterozygous tissues of celery (Apium graveolens L.) were introduced into culture, the incidence of altered phenotypes at these loci was monitored in association with karyological constitution. In the first experiment, progressive loss of allele expression over time independent of chromosome constitution was observed at one locus (Pgmm-2), while another linked locus (Sdh-1) retained the normal heterozygous phenotype. The variant phenotype was transmitted into regenerated plants, but these were sexually sterile. In the second experiment, phenotype at five independent isozyme loci were observed to be completely stable over 12 months in culture. After six months, cultured cells were mostly diploid, and plants could be regenerated readily. All phenotypes were, with one exception, completely heterozygous, and all plants checked (42 of 95) had normal diploid karyotypes, although two carried accessory or fragment chromosomes. After twelve months, the culture was completely aneuploid and failed to regenerate. It was concluded that gross changes in chromosome number and structure in cultured tissues are either the result of non-random events such that loci of adaptive importance are conserved, or that karyotypic changes are not generally representative of implied changes in the genotype at Mendelian loci.

147. Genetic Analysis of Alfalfa Variants from Somatic Cell Selection. E. T. BINGHAM, Department of Agronomy, University of Wisconsin, 1575
149. Development of an In Vitro Model for Toxicity
Several classes of variants have been regenerated from diploid alfalfa clone HG2 with and without mutagenesis and selection. The highest frequencies of variants and the only dominant genetic variants identified thus far were produced after mutagenesis in culture with methane sulfonic acid ethyl ester (EMS) and selection for resistance to ethionine, an analog of methionine. Plating unmutagenized cells on media containing ethionine resulted in more than 20% variant plants (2–3% in control), hence ethionine probably exaggerates the variability in experiments where it is used. Genetic analysis has focused on euploid diploid variants (polyplod and aneuploid variants were also recovered). Morphological variants controlled by dominant, recessive and non-genetically transmissible factors have been identified. Interestingly, some traits which behave as recessives in genetic analysis were directly identified in the diploid HG2 background, perhaps because the donor clone is well characterized and even slight modifications of phenotype can be detected. Spontaneous variants arising in untreated cultures have been fewer in number but often possessed significant changes, including changes in complex traits such as herbage yield. Comparisons of the best somaclonal variant and the best sexual derivative of HG2 will be reported.

148. Organotypic Tissue Culture Models of Neurotoxic Sensory-Motor System Diseases. P. S. SPENCER, R. B. Veronesi, J. Zagoren, M. Seelig, E. R. Peterson* and M. B. Bornstein,* Albert Einstein College of Medicine, Bronx, NY 10461
Organotypic cord-ganglia-muscle cultures have been used to reproduce in vitro specific types of neuronal and axonal degeneration found in animals and humans exposed to neurotoxic agents. Mature (8-week-old) cultures continuously exposed to 2.8 mM n-hexane or its metabolites (2-hexanol, 2-hexanone, 2.5-hexanediol, 5-hydroxy-2-hexanone, 2.5-hexanediol) displayed, after 4–6 weeks, the same pattern of giant axonal degeneration in distal motor and sensory nerves as seen in human and murine hexacarbon neuropathy. Methyl ethyl ketone (10–100 μg/ml), a compound that potentiates the neurotoxic action of n-hexane in vivo, caused a more rapid appearance of giant axonal degeneration in cultures exposed simultaneously to both solvents. Unlike methyl ethyl ketone, toluene or ethanol failed to alter the in vitro neurotoxic potency of n-hexane. Explants exposed for 4–6 weeks to methyl ethyl ketone alone (100 μg/ml), methyl isoamyl ketone (400 μg/ml) or 3-methyl hexane (1000 μg/ml), remained free of neuronal or axonal degeneration. None of these agents was able to induce neuroptathy in treated animals. By contrast, doxorubicin, a chemotherapeutic drug that induced sensory neuronopathy in treated rats, caused sensory neurons in vitro to undergo chromatolysis and degeneration within days of exposure to 10^{-6} M. In summary, sensory and motor neurons in organotypic combination cultures retain their selective vulnerabilities to neurotoxic agents.

149. Development of an In Vitro Model for Toxicity Testing of Cosmetic Formulations. W. H. J. DOUGLAS
No abstract received

150. Rat Kidney Epithelial Cell Culture to Study Metal Toxicity. M. GEORGE CHERIAN, Department of Pathology, University of Western Ontario, London, Ontario N6A 5C1, Canada
Most of the cellular toxic effects and adaptive changes on the kidney in metal toxicity occur primarily on the epithelial cells. We have undertaken studies on monolayer culture of primary epithelial cells from rat kidney to understand the mechanism of these processes. Cells were isolated from adult rat kidney cortex by mild trypsinization and maintained in a selective Eagle's MEM, supplied with D-valine instead of L-valine for 3 days to form a monolayer. In D-valine medium, fibroblast proliferation was inhibited because of the absence of D-amino acid oxidase in this cell type and an epithelial cell monolayer was formed. These cells were grown to confluency and provided an in vitro system to study the toxicity and cellular effects of metals (Cs⁺, Zn²⁺, Hg²⁺ and Pb²⁺). The induced synthesis of metallothionein (MT), an intracellular metal binding protein, in response to metals and its role in metal toxicity and detoxification were studied. Although the intracellular presence of MT was protective against cellular toxicity of Cd²⁺, its extracellular presence as CdMT was about 8 times more toxic than CdCl₂. The results suggest that a monolayer of rat kidney epithelial cells is an excellent system to study the toxicity and cellular adaptation to excessive exposures to environmental chemicals.
(Supported by grants from NIH (ES01535) and MRC, Canada.)

151. Altered Regulation of Proliferation and Differentiated Function in Cultured Human Epidermal Cells by Chlorinated Aromatic Hydrocarbons. W. F. GREENLEE, L. G. Hudson, W. S. Stillman, R. D. Irons and W. A. Toscano, Chem. Ind. Inst. of Toxicol., Res. Tri. Pk., NC 27709 and Harvard Sch. of Publ. Hlth., Boston, MA 02115
The proliferation and differentiation of epidermal cells is regulated by several biochemical mediators including hydrocortisone (HC), epidermal growth factor (EGF) and cyclic nucleotides. We have examined potential biochemical mechanisms by which 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) can alter the controlled growth and differentiation of squamous cell carcinoma (SCC) lines derived from human epidermis and tongue. In cell lines which exhibit an increased proliferative index in the presence of HC (1 μM), TCDD stimulated both colony expansion and keratinization. In the absence of HC, TCDD treatment resulted in small highly keratinized colonies, suggesting that a large portion of proliferating basal cells were committing to terminal differentiation. Proliferative responses were not associated with changes in EGF binding; however, in lines in which TCDD markedly inhibited colony expansion, the specific binding and uptake of EGF was decreased 2- to 3-fold. The observed potency (EGF = 1μM) and stereospecificity suggested that this response was mediated by the EGF receptor. The intracellular concentration of cyclic AMP was increased in cells showing a proliferative response to TCDD. The data indicated that TCDD can activate the adenylyl cyclase complex directly, apparently via a unique receptor system.
152. Genetic Toxicity Evaluation of Chemicals: Implications for Animal Assays. J. W. SPALDING

No abstract received

153. Vascular Smooth Muscle Cell Modulation in Vivo and Growth Potential in Vitro. J. GRUNWALD and C. C. Haudenschild*, Mallory Institute of Pathology, Boston University, School of Medicine, 784 Mass. Ave., Boston, MA 02118

Vascular smooth muscle cells (SMC) display two phenotypes. The normally quiescent or contractile SMC are characterized by extremely low division rates, large amounts of myofilaments, few mitochondria and little rough endoplasmic reticulum. In vivo these cells are typical of the normal vessel while in vitro this status can be maintained for a short time when the cells are harvested enzymatically. Activated or modulated SMC are characteristic of atherosclerotic lesions in vivo and of SMC obtained from explants in vitro. These cells have a high rate of proliferation and enhanced synthetic activity (as expressed by large amounts of mitochondria and rough endoplasmic reticulum) but few myofilaments. Balloon catheter de-endothelialization resulted in activated SMC in vivo. After 4 days these SMC exhibited the modulated morphology as seen by electron microscopy. Furthermore, the in vivo activation significantly changed their in vitro properties when cultured by explant technique. The explants showed SMC outgrowth after 24 hours and formed colonies of 118,000 cells per explant. Explants from control animals required 4 days for outgrowth resulting in 59,000 cells per explant. The number of explants showing any outgrowth was increased from 73% to 92% in 10% FCS, from 12% to 83% in 0.1% FCS and from 1% to 41% in serum-free medium. The total harvest of primary SMC per rat aorta could be increased up to 150% (5x10^6 cells). Second passage SMC continued to show a higher proliferation than SMC from control animals.

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154. Stimulation of Growth and Expression of Differentiation Antigens of Human Melanocytes by Tumor Promoters. M. EISINGER*, O. Marko, A. Houghton and I. B. Weinstein**, Memorial Sloan-Kettering Cancer Center and **Columbia University, New York, NY

In previous studies we found that TPA (10 ng/ml) when added to primary normal human epidermal cultures, selectively suppresses the growth of the otherwise predominant keratinocyte cell population; this is associated with the outgrowth of normal melanocytes. The present study indicates that these melanocytes can be grown subsequently for at least 30 passages if the medium contains TPA, but if the compound is removed the cells cease to divide. The ability of a series of phorbol esters to support the growth of normal human melanocytes correlates, in general, with their tumor-promoting activity on mouse skin. Two structurally unrelated types of compounds, teleocidin and aplysiatoxin, also support melanocyte growth. On the other hand, several polypeptide growth factors could not substitute for TPA. Utilizing a panel of monoclonal antibodies we have also found that some of these tumor promoters induce the expression in normal melanocyte cultures of antigens which are markers of early and intermediate stages of melanocyte differentiation. Since human melanoma cell lines grow vigorously in the absence of tumor promoters our results suggest that the malignant transformation of melanocytes is associated with the acquisition of autonomy from certain unidentified endogenous growth factors.

155. Biologic Modification of Antigenic Expression by Dibutyryl Adenosine 3'-5' Cyclic Monophosphate in Cultured Human Glioma Lines. M. B. SISTI, L. A. Pollock, B. H. Smith, and P. L. Kornblith*, Surgical Neurology Branch, NINCDS, National Institutes of Health, Bethesda, MD 20205

Cyclic nucleotides, which have been implicated in the control of glial division and differentiation, have been demonstrated to alter the morphology, growth rate, and immunologic recognition of treated cultured human glioma lines in tissue culture. This study presents the results of a multiparameter analysis of 30 human glioma lines to the exogenous administration of N602 dibutyryl adenosine 3'-5' cyclic monophosphoric acid (dBcAMP). Results of this investigation revealed a heterogenous morphologic response to dBcAMP in which 27/30 lines demonstrated varying degrees of enhanced cell process formation, increased contact inhibition, and a more prominent cytoskeleton. Tumor grade was found to correlate with morphologic response to dBcAMP with 30 percent of high grade gliomas responding in contrast to 80 percent of low grade tumors showing response. Growth inhibition, which ranged from 35 to 90 percent, was further pronounced in the presence of low serum media (<1%) at which point all morphologically responsive lines failed to grow with respect to controls. In vitro enhancement of the autologous humoral immune response was detected in 30 percent of treated lines including 3/30 lines which demonstrated no humoral immune response prior to treatment with dBcAMP in the microcytotoxicity assay. Electrophoretic analysis of purified plasma membranes of responding lines demonstrated quantitative changes in the protein composition of the membrane at 60,000 daltons suggesting changes in the antigenic determinant of the treated responding cultures.

156. Preservation of Cultured Pancreas Explants from Syrian Golden Hamsters. J. Resau, L. Marzella, J. Cottrell, and R. Jones, Department of Pathology, University of Maryland and MIESS, Baltimore, MD 21201

Exocrine pancreatic tissue consisting of both ducts and acinar cells has been maintained in explant organ culture (Resau et al., In Vitro, in press). Ultrastructural alterations in the explants include a marked stimulation of autophagy and crinophagy. The stimulation of these lysosomal catabolic processes correlates with the extent of explant viability in vitro. We are modulating lysosomal function in these explants to enhance the preservation of acinar cells. To this end we have tested the effects of cycloheximide (inhibitor of autophagy in vivo), secretagogues (presumed inhibitors of crinophagy) and low temperature (inhibitor of lysosomal fusion processes)
on explant viability. Secretagogues enhanced the short term viability of the explants by approximately 25%. Low temperature (20°C) enhanced the viability of the explant 5 to 6 fold for at least 72 hours as determined by morphometric analysis. Explants stored for 24 hours at 4°C before culture (2 days) were as viable as explants cultured routinely. The relationship of low temperature culture to enhanced long term survival of the explant and to lysosomal function is being explored.

157. Type I Alveolar Epithelial Cell Differentiation in Fetal Rat Lung Organ Explants. J. A. McAteer* and T. J. Cavanagh, Department of Anatomy, Indiana University School of Medicine, Indianapolis, IN 46223

The terminal differentiation of the highly attenuated type I alveolar epithelial cell (AEC) was observed in a morphological study of fetal rat lung (FRL) development in vitro. Fragments of FRL (18 days g.a.) were explanted to organ culture in medium F12K-5% NBS under various physical substrate conditions including conventional interface and submersion culture. FRL was also cultured fully embedded within hydrated collagen gel (HCG). Tissue survival and development was dependent on culture conditions. All explants exhibited type II cell differentiation. Only explants within HCG showed formation of attenuated cells similar to the type I AEC in vivo. Explant development was characterized by distention of presumptive alveolar spaces accompanied by thinning of "alveolar" septa. Moderately narrowed septa were lined by cuboidal cells, highly attenuated septa were lined by extremely attenuated type I-like cells. Type I-like cells possessed an enlarged perinuclear area that often contained deposits of glycogen, mitochondria and membranous organelles. Thin cytoplasmic extensions were devoid of membranous organelles. Pinocytotic vesicles were rarely observed. HCG embedded explants rarely showed central necrosis or evidence of epithelial degeneration. Capillary development was disrupted, and vascular elements coalesced at the point of convergence of septa. These observations indicate that attenuation of the alveolar epithelium occurred in the absence of influence from a developing alveolar capillary network. (PHS 5-87-RR-5371).

158. Hypermethylation of Albumin and Alphafetoprotein Genes in Nonexpressing Mouse-Rat Hybrid Cells. W. Church and J. Papaconstantinou*, Dept. of HBC&G, U.T.M.B., Galveston, TX 77550

We have shown that albumin and α-fetoprotein (AFP) gene expression is extinguished in mouse hepatoma x rat fibroblast hybrids, and that this regulatory event involves a transcriptional repression. Regulation of eukaryotic gene expression has been correlated with the extent of methylation of cytosine residues either in the gene or in the 5' flanking region. In this study we examine the possibility that methylation of the albumin and AFP genes may be a molecular mechanism for the extinction of expression of these genes in the somatic cell hybrids. High molecular weight DNA was extracted from cell lines JF1 (rat fibroblast), BWTG3 (mouse hepatoma), and hybrids BJ01, BJ50, and BJ140, and digested with the isoschizomers Hpa II and Msp I. Southern analysis was done using radioactively labeled mouse and rat albumin and AFP cDNA probes. In general, high molecular weight fragments were found in Hpa II digests, but not Msp I digests, indicating a greater extent of methylation in the nonexpressing cell lines. In addition to the 14 kb and 7.9 kb Hpa II al- bulin fragments common to JF1 and the hybrids, a 9.8 kb fragment appears in the hybrids which is not present in expressing cells. These data indicate that specific methylation may be attributed to the DNA of nonexpressing cells. With AFP probes, a 7.8 kb Hpa II fragment appears, common to the hybrids, JF1s and adult mouse liver but is not found in BWTG3. We conclude from these observations that methylation of cytosine residues in these two genes is correlated with their expression and may play a role in their regulation.

159. Initiation of Muscle-type Phosphorylase (PPL) and Phosphoglycerate Mutase (PGAM) Synthesis is Not Nerve-dependent. M. Davidson, T. Mongini, S. A. Foster, S. DiMauro, and F. Miranda,* Columbia University, College of Physicians and Surgeons, New York, NY 10032

In regenerating muscle culture, satellite cells proliferate and fuse to form multinucleated syncytia, thus recapitulating embryonic myogenesis. Muscle cultures of biopsies from patients with genetic defects of muscle isozymes (e.g. PPL, PGAM) are morphologically and histochemically normal, because the myotubes are not fully developed and contain non-muscle isozymes, characteristically abundant in immature muscle (Miranda et al., Neurology 30, 367, 1980). To determine whether nerve factors are required for synthesis of muscle-type PPL and PGAM isozymes, we grew normal human muscle in absence of nerve and embryo extract. Indirect immunocytochemistry with specific anti-muscle PPL isozyme antibody showed PPL antigen in myotubes. Cellulose acetate electrophoresis showed only non-muscle PGAM-BB in one week-old myotube cultures, but some PGAM muscle isozyme (MM) was present, in six-week old cultures. By eight weeks, myotubes had deteriorated, and PGAM-MM was no longer present. This indicates that nerve is not required for initiation of muscle-type PPL and PGAM synthesis, but may play a role in further isozyme maturation. Muscle culture analysis of the developmental pathogenesis of muscle PPL and PGAM isozyme deficiencies may, therefore, require the use of innervated muscle culture. (Supported by NIH Grants NS 18466, NS 11766 and the Muscular Dystrophy Association.)

160. Inhibitory Effects of Mouse Serum on Chick Muscle Cell Differentiation. R. B. Finley and C. L. Parker,* Atlanta University, Atlanta, GA 30314

Normal mouse serum is known to exhibit inhibitory effects on the differentiation of various cell types. In chick chondroblasts, it significantly lowers the incorporation of arachidonic acid into various phospholipids (Parker, et al., Biochim. Biophys. Acta 620: 142, 1980). This finding suggests a decrease in membrane fluidity which
might play a key role in cellular regulation and differentiation. Since cell membrane fusion is important for myoblasts to form multinucleated myotubes, the present study was undertaken to access the effect of mouse serum on muscle cell differentiation. Primary cell cultures were obtained from 11-day-old chick embryo thigh tissue. The treated cells were grown in complete medium containing 10% mouse serum. Within 3-5 h following the administration of the test sera, the cells began to accumulate lipid droplets. By 24 h, large amounts of lipid were evident, and with longer periods of treatment many of the cells became rounded and ultimately detached from the surface of the culture dish. The degree of myoblast fusion into multinucleated myotubes was greatly reduced. When myotubes were serum-treated at various times following fusion, the accumulation of lipid droplets decreased, however, spontaneous muscle contraction did not occur. The effect of mouse serum appears to be irreversible, while the application of equivalent concentrations of serum from other species exhibited responses much like control cultures. These findings further corroborate our earlier suggestion that mouse serum may be modulating cell regulation and/or differentiation through alterations in the phospholipids of the cell membrane. (Supported by NSF Grant No. PRM-8111221.)

161. Reversible Inhibition of the Accumulation of Muscle-Specific Proteins by the Differentiation Inhibitor (DI). M. J. Ewing-Hodges, J. R. Florini*, and B. M. Vertel, Biology Dept., Syracuse Univ., Syracuse, NY 13210

Two years ago at these meetings we announced our discovery that Coon’s Buffalo Rat Liver (BRL) cells secrete a protein that is a potent inhibitor of myoblast differentiation. We now report that DI has dramatic and reversible effects on accumulation of muscle-specific proteins by myoblasts in culture; these have been demonstrated 1) by double immunofluorescence utilizing antibodies directed against myosin heavy chain (from Dr. D. A. Fischman), myomesin (from Dr. E. Stehler), and type I collagen (B. M. V.), and 2) by enzymatic assay of creatine kinase (from Dr. T. Parker,* Department of Biology, Atlanta University, Atlanta, GA 30314)

163. Characteristics of Chick Embryos Maintained in Shell-less Culture. G. B. Lester and C. L. Parker,* Department of Biology, Atlanta University, Atlanta, GA 30314

The development of chick embryos in vitro has been shown to proceed at a significantly slower rate than that of embryos cultured in ovo. Lower embryonic survival rates, produced when using culture vessels of low oxygen permeability, may be related to the decreased rate of development. This study was, therefore, conducted to determine if embryos cultured in vitro experience hypoxic-like conditions. Fertilized chicken eggs were precultured for approximately 72 h at 37-38°C and 60% relative humidity. The embryos were then removed from their shells, placed in a cup-type culture vessel and incubated in air at 37-38°C and 75-85% relative humidity. Embryos were removed from the incubator at various times to determine red blood cell counts and hematocrits. The embryos were also weighed and staged to determine growth and development during the 19-day culture period. During the last 10 days of culture, the developmental stage and weight of embryos cultured in vitro became increasingly lower than those of embryos cultured in ovo. In contrast, the hematocrit of embryos grown in shell-less culture became significantly higher than those of embryos cultured in ovo. The red blood cell counts increased linearly with the hematocrit. The data presented here show that embryos cultured in vitro acquire characteristics similar to those reported for embryos cultured in ovo but under hypoxic conditions. The data further suggest that the failure of in vitro cultured, whole chick embryos to fully develop and survive may be related to less than optimal oxygen levels. (Supported by NSF Grant No. PRM-8111221.)

164. Regulation of Differentiation of Adult Human and Rat Hepatocytes Cultured in a Serum-Free
Medium by Interaction with Another Liver Cell Type. C. GUIGUIN-GUILLOUZO*, B. Clement, D. Glaise and A. Guillouzo, Unité de Recherche Hépatologique U 49 de l’INSERM Hôpital Pontchaillou 35011 Rennes Cédex, France

Even if improving cell survival, no media or substrates of extracellular matrices prevent rapid phenotypic changes in cultured adult hepatocytes. We postulated that in vitro instability of hepatocytes was due to lack of specific in vivo cell-cell interactions. This hypothesis was tested by culturing adult human and rat hepatocytes isolated by enzymatic perfusion in association with various cell types. When these hepatocytes were co-cultured with undifferentiated rat liver epithelial cell lines (in opposition to human liver fibroblastic cells or non-hepatic epithelial cells), cell survival rate reached at least 2 months and high levels of specific functions persisted, even in a serum-free medium containing hydrocortisone. Close cell-cell contacts were required and were rapidly followed by secretion of an heterogeneous extracellular material rich in type III collagen, found primarily between the two cell types. The rat liver epithelial cells should be used before spontaneous transformation.

This suggests that simulation of in vivo conditions (tissue-specific cellular cooperation) is necessary for in vitro maintenance of hepatocytes phenotypic expression, with production of an “extracellular matrix”.

165. Histotypic Expression of Tumor Architecture by Human Cell Lines in Chick Embryonic Tissue Organ Culture. J. RIDGE, P. D. Noguchi,* Division of Biochemistry and Biophysics, Office of Biologies, National Center for Drugs and Biologies, Bethesda, MD 20205

One goal of organ culture methodology is to induce human tumor cell lines to reexpress original tumor architecture. Cell suspensions of the colon tumor lines WiDr and LS174T were inoculated onto agar discs surrounded by fluid medium with and without additional chick embryonic tissues (CET) and cultured for 3 to 45 days. When grown without CET the cells form actively growing masses with slight to moderate gland formation. With CET, however, the tumor cells organize within 7 to 10 days into miniature tumors with basal lamina and glandular formations that strongly mimic the in vivo tumors. Immunoperoxidase staining for carcinoembryonic antigen with monoclonal antibody showed that the expression of CEA increased over time for the WiDr cells, and seemed to be localized to the glandular areas. In contrast, LS174T cells grown on agar showed marked cytoplasmic staining both at early and late stages; when grown with CET the cytoplasmic staining was markedly reduced and glandular staining became much more apparent. These preliminary results suggest that extended organ cultures may be a useful way to study human tumor biology. [Supported by a grant from The American Federation for Alternatives to Animal Research (J. R.). Submitted in partial fulfillment of the Ph.D. requirements in Genetics, The George Washington University (J. R.).]

166. Comparison of Culture Media for the In Vitro Clonal Growth of Human Primary Breast Carcinoma Cells. G. J. BESCH, P. R. Rosenbaum, W. H. Wolberg and M. N. Gould*, University of Wisconsin, Wisconsin Clinical Cancer Center, Department of Human Oncology, Madison, WI 53792

The purpose of these experiments was to optimize media for the clonal growth of monodispersed primary human breast carcinoma cells in agar. Two media currently used for cultivating primary cancer cells (A,B) and modifications of them (C,D,E,F) were compared. The media were: A) Modified Eagle’s medium supplemented with fetall bovine serum (FBS), insulin, prolactin, progesterone, β-estradiol, and cortisol (Cancer Res 40: 1836, 1980); B) Ham’s F-12 and Dulbecco’s Modified Eagle’s media (1:1) supplemented with media conditioned by cultured human cells, FBS, insulin, EGF, T3, β-estradiol, cortisol and cholera toxin (Cancer Res 41: 4637, 1981); C) medium A without hormone supplementation; D) medium A without FBS; E) medium A without FBS and hormone supplementation; and F) medium B without conditioned media. The highest plating efficiencies (PE) and largest average colony diameters were obtained with medium A (PE=0.197%) and medium B (PE=0.173%). All other media had significantly lower (P<.05) plating efficiencies (C=0.096%; D=0.016%; E=0.004%; and F=0.088%). In other experiments, we tested 5-bromo-2’-deoxyuridine (BUDR), an agent associated with the alteration of cellular differentiation, for its ability to modify the clonal growth of primary breast carcinoma cells in vitro. We found that 1.0 and 5.0 µg/ml of BUDR significantly increased (P<.05) the plating efficiency of these cells, but higher concentrations were toxic. Supported by NCI Grant CA 20432.

167. Image Analysis in the Quantitation of Human Glioma-Derived Cell Properties and Chemotherapeutic Sensitivity. B. H. SMITH, J. R. Ellist, C. Cooke, C. Gibson†, C. Pepin, B. M. Chronwall, P. L. Kornblith*, M. A. Greenwood, and P. E. McKeever. Surgical Neurology Branch, NINCDS; †Division of Research Services, Biomedical Engineering & Instrumentation Branch; National Institutes of Health, Bethesda, MD 20205

For quantitative studies of the subpopulation properties and chemotherapeutic sensitivities of human glioma-derived cells in culture, we have interfaced a modified Bausch and Lomb Omnicon (FAS II) image processing system to an inverted light microscope with a motorized stage for automated micrometer plate cell counting and a JEOL JSM 35C scanning electron microscope for quantitation of cell morphology and surface properties. For the microtiter plate-based chemotherapy assay, the system generates reliable cell counts as well as relevant statistics in 15 minutes (vs 60 minutes manually). The scanning EM system permits rapid (5-10 min.) collection of up to 4 simultaneous geometric parameters in 100 or more cells of control or treated populations. One to 5 subpopulations have been detected in individual cell lines, with area and length-to-breadth ratio (L/B) being the best interline discriminators. Gial fibrillary acidic protein (GFA+) and fibronectin (FN+) cells migrating out of glial tumor explants in passage 0 can be discriminated utilizing area (4:1 FN+/GFA+); L/B ratio, (FN+, 1=3; GFA+, 5-10); and perimeter. AZQ (aziridinylbenzoquinone) sensitive populations (72 hr.
exposure 10 μg/ml show marked shrinkage (loss of area 2-10 fold) and rounding (L/B ratio: 3.0, control; 1.0, treated). Drug-resistant populations are defined.

168. Characterization of Cell Cultures from Normal and Neoplastic Human Brain Tissue. B. P. Barna*, J. W. Bay, S. M. Chou, G. A. Hoeitge, and B. JACOBS, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44106

Cell cultures were established from normal (uninvolved) and neoplastic (astrocytoma, grade IV) brain tissues from a female patient. Portions of cultures were cryogenically stored at passage 2 or characterized between passages 2 to 5. All cultures were mycoplasma-free. Cultures from both uninvolved brain tissue (UBTC) and astrocytoma tissue (ATC) were morphologically heterogeneous. Cells in UBTC were mostly polygonal or bipolar, with occasional multinucleated giant cells resembling "reactive" astrocytes. Most ATC cells were astrocytic in appearance, with long, narrow processes, but numerous multinucleated giant cells were also present. Treatment with dibutyryl cyclic adenosine monophosphate (cAMP) produced elongated cytoplasmic processes in ATC and vacuolated cytoplasm in UBTC cells. By immunoperoxidase staining, both cultures were found to have cells containing bovine IgG, apparently from the culture medium, as well as occasional cells containing glial fibrillary protein. Mean doubling time was 111.2 hours for UBTC and 54.3 hours for ATC. ATC demonstrated a pseudo-diploid karyotype (46, XX, del(7q21q22), +7, −10) in approximately 1/3 of the cells, while the remainder had the same basic karyotype but were 4n or 6n. These preliminary observations indicate that characterization of normal and neoplastic brain cultures is complicated by cellular heterogeneity, and that further work should be directed at separation of cell subpopulations.

169. Inhibitory Effects of Auranofin† Analogs on HeLa Cells. T. M. SIMON*, D. H. Kunishima, J. D. Hoeschele, A. Lorber, Memorial Hospital Medical Center of Long Beach, Long Beach, CA 90801

The parent coordinated gold compound, Auranofin (AF), has demonstrated antiproliferative properties against several established cancer cell lines and primary (soft agar) tumor cultures. Accordingly, three analogs of AF were synthesized to determine if they also possess antineoplastic activity. The analogs differ from AF by containing group exchanges i.e., methyl, isopropyl and phenyl, in the triethylphosphine moiety of the molecule. Using DNA synthesis as reflected in macromolecular incorporation of tritiated thymidine. Incorporation of tritiated thymidine. The parent compound and three analogs were tested at 25, 50 and 100 μg/dl (gold content), and exposure times ranged from 2-24 hrs. A 2 hr exposure at 50 μg/dl resulted in an 86% decline in isotope uptake from untreated control cultures for the AF, and an 82%, 85%, and 93% decline for the methyl, isopropyl and phenyl analogs respectively. Longer exposures of 24 hrs resulted in a >95% reduction in isotope uptake. These declines were observed to be concentration dependent. Morphological changes associated with treatment included surface membrane pitting, blebbing, and cell rounding with detachment. These observations suggest that this new class of gold compounds may have application as antineoplastic agents.

†Smith Kline Beckman (2,3,4,6-tetra-0-acetyl-1-thio-β-D-glucopyranosato-S (triethylphosphate) gold.

170. Intrinsic and Acquired Resistance to BCNU Correlates with the Near-Diploid Cells in 4 Freshly Resected Human Gliomas. J. R. SHAPIRO*, P-Y. Pu, W. R. Shapiro, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

We have previously demonstrated that human malignant gliomas are karyotypically heterogeneous and that different clones from a patient's tumor differ in their chemosensitivities. Preliminary observations also suggested that clones with near-diploid chromosome numbers with 45-49 chromosomes were more resistant to drugs than were hyperdiploid clones with 55-89 chromosomes. To examine this observation in greater detail, we tested 4 human gliomas and their clones by colony forming assay (CFA) in monolayer tissue culture using BCNU and compared the chemosensitivities to the tumors' chromosome numbers. All 4 tumors were karyotypically heterogeneous; chromosome numbers ranged from hypodiploid to near-tetraploid. CFA on the parental tumors showed that 1 of the 4 was sensitive to BCNU at 5.0-7.5 μg/ml while 3 were resistant at doses of 15 μg/ml or greater. A total of 20 clones in 3 of the 4 tumors were sensitive to BCNU at 2.5-7.5 μg/ml. All but two of these clones had a chromosome number greater than 51 per cell; the other two were hypodiploid having less than 39 chromosomes. Twenty-three near-diploid clones with 45-47 chromosomes were isolated and all but one of these were resistant to BCNU. Thus, BCNU resistance was associated with near-diploid chromosome numbers while BCNU sensitivity was found either in hyperdiploid cells having 55 or more chromosomes or in hypodiploid cells. Combined with our previous findings that near-diploid and not hyperdiploid clones can develop resistance to low-dose BCNU, it appears that the near-diploid cells in gliomas are ultimately responsible for both intrinsic and acquired resistance to BCNU chemotherapy.

171. Use of Athymic Nude Mice to Establish Human Tumor Cell Lines. J. W. HARBELL*, D. B. Mercill and L. L. Woods, Fitzsimons Army Medical Center, Aurora, CO 80045

The study of human tumor cells in vitro has been hampered by the inability to establish cultures from many clinical samples. This difficulty often results from the small size or low viable tumor cell fraction. Therefore, a means to increase the number and proportion of tumor cells sample would be of value if such a mechanism did not alter the characteristics of the proliferating populations. To this end, we have implanted samples directly into athymic nude mice and compared the resulting nude passage derived cell lines with those obtained by direct culture. In unaltered mice, the percentage of samples which grew was low, however, when the recipients were splenectomized and treated with antilymphocyte serum (ALS) 58% (n=39) of primary implants from 14 tumor types were successful. Of those harvested for culture,
61% yielded cell lines against a 38% success rate for the same samples taken directly from the patients (n=13). Three direct and nude derived line pairs have been compared in detail. In all cases cell morphologies, growth rates and saturation densities were similar. Chromosome analysis indicated no differences in modal number or marker chromosomes. Sensitivities to a panel of 15 chemotherapeutic agents were not significantly different. These data suggest that (1) the splenectomized, ALS treated athymic nude mice may provide a useful means to increase the number of samples (2) that lines derived from those implants would be similar to those which would be derived directly.

172. Control of Growth and Differentiation in Rat Prostatic Adenocarcinoma Cells by Butyrate and Related Short-chain Fatty Acids. D. H. REESE, N. L. Block, V. A. Poltano, Department of Urology, University of Miami School of Medicine, Miami, FL 33101

Retinoic acid (RA) has recently been shown to cause the RNA- and protein-synthesis dependent induction of alkaline phosphatase (AP) activity in normal and malignant rat prostatic cells and in rat bladder urothelium. As one of several approaches we are taking to define the molecular mechanism by which RA induces AP, we have investigated other potential inducers of the enzyme. Using the retinoid-responsive 9-1C cell line, a rat prostatic adenocarcinoma line derived from the Dunning R3327 tumor, butyrate (BU) was also found to induce AP. Enzyme activity increased linearly between 2 mM and 10 mM BU. Growth was also inhibited in this concentration range; 1.5 mM and 5 mM caused 50% and 100% inhibition respectively. Growth inhibition was reversible, with normal growth rates resuming within 24 hrs after BU (3 mM) removal. Flow cytometry measurements showed that, beginning with 3 mM, cells got progressively larger with increasing BU concentrations. Cell protein content also increased; 4 mM BU caused a 4-5 fold increase in cell protein. Although this latter observation was unique to BU, other short-chain fatty acids (propionic, valeric and caproic) also caused significant suppression and AP induction. BU dramatically altered cellular growth patterns. The cells, which normally grow in a disorganized array, were organized into clusters or oriented end-to-end into tracts and whirls through the monolayer.

173. Effect of Gonadal Hormones (GH) on Human Renal Cell Carcinoma (RCC) in Serum-Free Medium (SFM). A. L. BEAR, R. V. Clayman*, R. S. Figenshau, E. E. Fraley, University of Minnesota Hospitals, Department of Urologic Surgery, Minneapolis, MN 55455 (Sponsored by R. V. Clayman)

Given the presence of hormonal receptors in RCC and occasional clinical responsiveness to progesterone (PG) therapy, we sought to examine the effect of GH on the growth of RCC lines 786-0 and Caki-1 in SFM developed by Taub. Growth curves with triplicate counting points on days 3, 6, and 9 were taken under 4 conditions: 1. SFM 2. SFM+PG 3. SFM+10% Fetal Calf Serum. We also studied the response of 2 RCC lines (TK-10 and 769-P) which previously failed to grow in SFM. To determine optimal conditions for each line, varying concentrations of PG and DHT were added from physiologic to 60x physiologic levels. Growth of 786-0 was significantly inhibited by physiologic levels of DHT. In contrast, neither hormone affected the growth of Caki-1. TK-10 and 769-P also showed no change in growth with DHT or PG present. For optimal growth in SFM, components must be customized to the particular cell being cultured. PG and androgen receptors in RCC may likewise indicate the requirements of these cells for GH, which seems to be the case for 786-0 but not the other 3. Relationship of these hormonal receptors to the tumors' growth potential is currently under study. Development of SFM in which lines of RCC can be propagated is also of value for studying those biochemical alterations resulting in its clear cell appearance.

174. Differential Phenotypic Response of Normal and Neoplastic Mouse Mammary Epithelium in Collagen Matrix Culture. W. JONES, A. Lee, R. C. Hallowes, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, U.K.

The BR6T mouse strain is a useful model to investigate mammary disease, particularly since the strain is susceptible to benign (pregnancy-dependent) and malignant forms of neoplastic disease, each having a counterpart in the human (carcinoma in situ, primary carcinoma). Diseased mammary glands from mice bearing these tumors were isolated and subjected to a limited collagenase digestion. Complementary non-involved glands from the same animals were taken for controls. The released tissue was passaged through a graded series of filters and the multicellular fractions collected upon each of the filters, along with the filtrate fraction (<31μ), were established in culture within a collagen matrix. The normal mammary epithelium in both control and tumor cultures responded in a characteristic manner in culture. Tumor cell clusters from the pregnancy-dependent tumor displayed a lichen-like morphology. Spontaneous-tumor clusters remained as rounded masses which occasionally extended broad, blunt projections into the gel, subsequently retracting, leaving behind spherical satellite bodies. Filtrate fractions from these tumors did not display this response. The results of these studies demonstrate the usefulness of the collagen matrix culture system for distinguishing between different pathological forms according to their phenotypic behavior within the gel. Further, the results indicate the importance of maintenance of the cellular microarchitecture of tumor clusters for their ability to display their unique phenotypes in collagen gel. (Sponsored by L. Anderson*).

175. Bromocriptine Acts As an Agonist-antagonist of Angiotensin in Human Adrenocortical Conn's Adenomatous Cells in Primary Culture. U. ARMATO* and F. Mantero, Depts. of Human Anatomy & Internal Medicine, Univ. of Padua, I-35100 Padua, Italy

Dopamine's role in the control of aldosterone secretion is still debated; though this process is stimulated by metochlorpromide (a dopamine antagonist) and inhibited by
bromocriptine (BC, a dopamine agonist), it remains unclear whether these agents act at the adrenal level or elsewhere. To shed light on this topic, we used primary cultures of human adrenocortical Conn's adenomatous cells (1), i.e. of a benign neoplasia producing high quantities of aldosterone. Our preliminary finding show that both angiotensin (AT) and BC, when added each by itself daily at 10^{-8} \text{ mole/l}, stimulated the de novo RNA and protein synthesis of Conn's cells during the following 5 days. However, when used in equimolar mixtures, AT and BC left unchanged the basal rates of new RNA and protein synthesis with respect to the untreated control cells. Neither AT nor BC, used singly or in equimolar association, influenced the flow of Conn's cells into DNA synthesis or mitosis. Therefore, we surmise BC acts as the adrenal cell layer by interfering with the AT receptor sites according to a partial agonist-antagonist mechanism of action depending upon the concentration of BC at the cell membrane and the presence of absence of AT.

(1) Andreis, P. G.; Mantero, F.; and Armato, U. Pathol. Res. Pract. 173, 66 (1981).

176. Effect of Dextran and Ficoll on Enhancement of Antibody Production by Murine Hybridoma. N. YABE, Y. Matsuya and I. Yamane*, Research Institute for Tuberculosis and Cancer, Tohoku University, Sendai 980, Japan

The efficiency of cell hybridization is usually enhanced by agglutinating cultured cell lines with lectin before fusing cells with polyethylene glycol. When this technique was used in murine hybridoma formation, the efficiency of hybridization was not promoted; furthermore lectins markedly decreased cell viability, although myeloma cells and spleen cells (sensitized with C-reactive protein as immunogen) formed cell clumps after lectin treatment. Therefore, we investigated other agglutinins and found that Dextran and Ficoll agglutinate cells and are less cytotoxic. Treatment with these polysaccharides did improve hybridization, but the ratio of antibody produced by hybridoma was two times that of untreated cells. Thus higher frequencies of specific hybridoma formation was quite reproducible. The conditions of Dextran treatment were as follows: average molecular weight was \(4 \times 10^{5} \text{-} 5 \times 10^{5}\); concentration, 0.5-10%; exposure time, 10-30 min. Although the mechanism of this effect is not yet clear, it offers a basis for an effective method for preparing specific antibodies by the hybridoma technique.

177. Characterization of Monoclonal Antibody Produced Against Varicella Virus. C. Clegborn, J. P. Ilitis, D. L. MADDEN, A. K. Weinrod, D. A. Fuccillo, N. I. and Microbiological Associates, 5221 River Road, Bethesda, MD 20814

Hybrid cells secreting mouse monoclonal antibodies against varicella virus were produced. Varicella antibodies were characterized for immunoglobulin class, ability to neutralize virus, react in an indirect fluorescent membrane antigen test (FAMA), fix complement, perform in an enzyme-linked immunoabsorbent test and to detect antigen using the gel immunoprecipitation technique. Based upon these studies 5 groups were identified. The cells from these groups were recloned and representative antibodies from each were studied in detail. Group 1 was an IgG2ak with minimal FAMA and high ELISA (32,768 to 524,288) titers, fixed complement and neutralized virus. Group 2 was an IgG2bk had FAMA titers and fixed complement. Group 3 was an IgG2k with high FAMA and low ELISA titers (32 to 128, 256 to 16,384 respectively), did not fix complement and most did not neutralize virus. Group 4 was an IgMk with minimal FAMA and medium range ELISA (4098-32,768) titers, did not fix complement and some neutralized virus. Group 5 was a very heterogenous group, mainly IgG2ak and IgM, all reacted in FAMA and some fixed complement. Using \(^{35}\text{S}\) and \(^{3}\text{H}\) labeled cell lysates, normal antibody positive serum precipitated 14 to 17 proteins. Immunoprecipitation tests indicated that major differences existed in monoclonal antibody produced.

178. Polyclonal and Monoclonal Antibodies that Identify Breast Myoepithelial and Breast Fibroblast Cells. R. L. CERIANI, E. W. Blank and J. Lee, Bruce Lyon Memorial Research Laboratory, Children's Hospital Medical Center, Oakland, CA 94609. (Supported by J. A. Peterson*)

We had already identified breast epithelial cells with heterologous antisera (anti-HME) (Proc. Nat. Acad. Sci. 74: 582, 1977) and presently developed polyclonal (anti-F) and monoclonal (McF) antibodies against differentiation antigens of breast fibrocytes and polyclonal antibodies (anti-myo) against myoepithelial cells. They were prepared by immunization with human breast fibroblasts and myoepithelial cell line HS578Bst (J. Nat. Cancer Inst. 58: 1795, 1977) respectively. After absorption anti-F stained with specificity by immunofluorescence (IF) fibroblasts and the cells in the stroma around breast alveoli. McF stained specifically fibroblasts in monolayers. Strand-like structures overlaying HS578Bst cells in monolayers and cell surface aggregates in suspended cells were detected by anti-myo by IF. In tissue sections anti-myo stained by IF cells closely attached to the alveolar cells and their prolongation among the epithelial cells. Anti-F, McF, anti-myo and anti-HME are useful in identifying the different cell types of the breast after cell dispersion and monolayer culture and to identify different cells when pathological conditions alter normal morphology of the gland. Supported by ACS grant PDT-989.

179. Trypanosoma cruzi Infection of Human Muscle Cells is Inhibited by Antibodies to Parasite Surface Antigens. R. BOROJEVIC*, J. Scharfstein, L. Mendonça-Prevital, Institut Pasteur, 69365-Lyon, France and Universidade Federal, Río de Janeiro, Brazil

The intracellular parasitism of human cells by the pathogen T. cruzi is limited to a few cell types (muscle and nerve cells, macrophages), depending on the parasite strain and host immunity. A new T. cruzi surface glycoprotein antigen of 25000 MW (G25) was recently isolated; antibodies to G25 (aG25) were affinity-purified from Chagas' disease patients' sera, or obtained from hyperimmunized rabbits. Primary cultures of human smooth muscle cells and fibroblasts were infected in vitro
180. Production of the C Component of Complement by Cultures of Rat Liver Epithelial Cells. Stimulating Effect of Supernatants of Human Blood Cells. M. GUIGUET* and G. Mack, INSERM U 208, Faculté de Médecine, 21033 Dijon, France
A rat liver epithelial cell line grown in 10% serum HamF10, Mo 6FR, has been investigated for complement C3 component biosynthesis between the 4th and the 10th passage after primary explantation. This cell line was found constantly diploid, using G-banding karyotype analysis. After a 32 h incubation with small aliquots of medium supernatant of human blood cell culture added to fresh culture medium, a 6 to 10 fold increase in the production of this complement protein was observed. Human blood cells were cultured during 72 h in the same Ham F10 medium. When blood white cell culture medium supernatant was submitted to either heat or extreme pH or also trypsin digestion, the stimulating effect was strikingly reduced or even suppressed. This suggests that the stimulating factor(s) corresponded to protein(s). Details will be given on the RIA of this complement component using purified rat C3 antigen, the biosynthesis rate and the lymphocytic origin of the stimulating factor(s).

181. Preparative Density-Gradient Electrophoresis of Cultured Human Embryonic Kidney Cells. M. E. KUNZE, L. D. Plank, P. Todd*, and V. Giranda, Althouse Laboratory, The Pennsylvania State University, University Park, PA 16802
The mammalian kidney contains cells that transport water, convert vitamin D to active forms, synthesize hormones such as renin and erythropoietin, and produce enzymes such as urokinase, a plasminogen activator. Biochemical study of these functions in their individual cell enzymes such as renin and erythropoietin, and produce enzymes such as urokinase, a plasminogen activator. Biochemical study of these functions in their individual cell.

182. Collagen as an Attachment Factor for Cell Monolayers on Gas-Permeable Teflon Membranes. M. G. GABRIDGE* and M. F. Glass, W. Alton Jones Cell Science Center, Old Barn Road, Lake Placid, NY 12946
The ability to cultivate cell monolayers on gas-permeable synthetic membranes presents a distinct advantage for evaluating the cytotoxicity of gaseous compounds. Though some cell types will attach readily to the membranes, fibroblasts of respiratory tract origin show a low attachment efficiency on such a surface. We evaluated the ability of several soluble collagens to promote membrane attachment of lung fibroblasts. Etched, FEP-teflon membranes were autoclaved in situ in “Chamber/Dishes” with a 1.5 ml (25 mm diam.) chamber. Membranes were treated for 10 min. with acid soluble collagens (mouse tendon or calf skin). MRC-5 cell attachment and viability were quantitated after 30 hrs through ATP measurements made with a luciferin/luciferase system. Collagen increased attachment by 2- to 5-fold. Other treatments such as poly-L-lysine, BSA, and fetal bovine serum were modest and variable in effect. Other cell types (HeLa and HEP-2) attached better to membranes than did MRC-5 cells, regardless of pretreatments. Acid soluble collagen was the most effective promoter of lung fibroblast attachment to gas-permeable synthetic membranes, and increased monolayer ATP content from a control level of 2.48 ng to 5.76 ng after 30 hrs in culture. It should prove helpful for use in cell systems where flexible membranes offer technical advantages such as: (1) maintaining cells which require high oxygen levels; (2) sectioning for transmission electron microscopy; and (3) evaluating gaseous pollutants for toxic potential.

183. Large Scale Culture of Human Breast Carcinoma Cells to Isolate Estrogen Receptors. M. T. WININGER*, W. D. Ross*, T. Allen*, Monsanto Research Corporation, Dayton, Ohio; University of Louisville, Louisville, KY
Human breast carcinoma cells, line MCF-7, have been cultured in large-scale suspension culture to isolate estrogen receptors. MCF-7 cells were first adapted to grow in monolayers in Modified Dulbecco’s medium with high glucose and 5% fetal bovine serum. Cells grown in suspension culture using this medium grew to higher densities and had higher viability when compared to cells grown in MEM with 10% calf serum normally used for monolayer culture. To initiate suspension cultures, partially dissociated cell aggregates from monolayers were inoculated into small (300 ml) suspension culture flasks. Cultures were then progressively scaled up to multiple 20 liter culture bottles. Cell densities as high as 3.8 ml packed cells per liter of culture were obtained. One culture series was continuously exposed to high concentrations of insulin to evaluate further production of estrogen receptors. In contrast to results obtained in monolayer cultures, added insulin did not stimulate higher receptor levels. Receptor levels were determined to be 50 to 100 femtomoles per mg of cells.
184. An Assay for the Accuracy of Protein Synthesis in Red Blood Cells. K. D. Tschanz, A. Gotto, and C. L. BUNN. Department of Biology, University of South Carolina, Columbia, SC 29208.

The accuracy of translation in protein synthesis is measured as the rate of misincorporation of a particular amino acid different from that specified by an mRNA codon into protein. In vivo measurements have shown error rates of 1 in $10^{10}$ amino acids. In vitro measurements have used synthetic mRNAs, and error rates of 1 in $10^5$ amino acids have been reported, which are too high to be physiologically significant.

The cowpea variant of tobacco mosaic virus, cTMV, contains no cysteine or methionine in its coat protein. Translation in vitro of purified cTMV coat protein mRNA by rabbit reticulocyte lysates under optimal conditions has been performed, and the coat protein product purified by immunoprecipitation with specific antisera. The total amount of coat protein synthesized was measured by $^3$H-leucine incorporation, and the translational error rate was measured by comparing the incorporation of $^{35}$S-cysteine into purified coat protein, with the total synthesis of this product. An error rate of $10^{-6}$ cysteine molecules per amino acid incorporated was obtained, which compares well with in vivo measurements. (Supported by NIH grants AG02664 and 2507 RR7160, and a grant from the Elsa U. Pardee Foundation, all to C.L.B.)

185. Evaluation of a New and Flexible Method for Culturing Macrophages: Attachment to and Removal from Cytodex. Microcarriers. C. Ostlund, J. Clark, M. KRUSE. ‘Cell Biology Group, Pharmacia Fine Chemicals, Box 175, Uppsala, Sweden; 2Pharmacia Fine Chemicals, Div. of Pharmacia Inc., 800 Centennial Avenue, Piscataway, NJ 08854.

Various microcarriers were studied for the high yield culture of macrophages. Mouse peritoneal macrophages were cultivated on Cytodex 1, Cytodex 2, (positively charged surfaces) and Cytodex 3 (denatured collagen coated surface). The kinetics of attachment depended on the type of microcarrier surface, suggesting different mechanisms of attachment. Macrophages attached more rapidly to both Cytodex 1 and Cytodex 2 with more than 85% of the inoculum attached after 1 hr. The rate of attachment to Cytodex 3 was slower and after 2 hrs. 78% of cells attached. Maximum attachment depended on using the correct spatial distribution of microcarriers. L-cell conditioned medium was necessary for maintaining high cell numbers for prolonged periods. Macrophages cultured on Cytodex retained their ability to phagocytize latex particles. Trypsin treatment of macrophages attached to Cytodex 3 resulted in recovery up to 100% and >95% viability.

The important advantages of using microcarriers were: 1) a manipulable culture system which can be used in assays, interaction systems or as feeder cultures 2) providing a culture surface which facilitates attachment of macrophages and easy removal without loss of viability.

186. Isolation of Epithelial Subcomponents of the Mouse Mammary Gland for Tissue-Level Culture Studies. W. JONES, N. Choongkittaworn, and H. L. Hosick. Department of Zoology, Washington State University, Pullman, WA 99164

The unique physiology and pathobiology of the terminal epitelial region of the mouse mammary gland have been poorly studied at the tissue level in culture, due partly to inadequate tissue isolation procedures. We describe a simple and efficient method for the isolation of the epithelial subcomponents of the mammary gland for tissue-level culture studies. Whole mammary fat-pads are coarsely minced and subjected to a limited collagenase digestion, with physical disruption, for 90 minutes at 37°C. The adipose cells are mostly disintegrated by this procedure. The parenchyma is released as a mixture of multicellular organoids and monodispersed cells. Use of a graded series of filters allows the separation of parenchyma from non-parenchymal material, with a further enrichment of the former into ductal, ductal-lobular, and terminal end-bud or alveolar populations. The use of a number of filters within a selected size-range (400, 250, 150, 95, and 51 microns pore-size) provides a further sorting of the organoids according to size. Yield is variable and dependent upon the nature of the starting tissue, e.g., mouse strain, age, and parity. Preservation of the structural integrity of the glandular epithelium aids in the maintenance of its differentiated function in culture. The ability to provide discrete ductal and end-bud populations for tissue-level culture studies is important in our efforts to understand the nature of the differences in growth pattern and function between the two regions of the gland during adolescence, pregnancy, and disease. (Supported by NIH grant AG-02909).

187. Cytotoxicity and Absence of Mutagenic Activity of Vomitoxin (4-deoxynivalenol) in a Hepatocyte-mediated Mutation Assay with V79 Chinese Hamster Lung Cells. C. G. ROGERS and C. Héroux-Metcalf. Toxicology Research Division (Foods, Health and Welfare Canada, OTTAWA, K1A 0L2

Vomitoxin or 4-deoxynivalenol (3,7,15-trihydroxy-12,13-epoxytricothec-9-en-8-one), is a tricothecene mycotoxin produced on cereal grains by Fusarium graminearum and Fusarium roseum. Among 40 known kinds of tricothecenes, vomitoxin is one of the dominant pollutants of cereal grains, e.g. winter wheat. We examined the cytotoxicity and mutagenicity of purified vomitoxin in vitro in an hepatocyte-mediated mammalian cell mutation assay with V79 Chinese hamster lung cells as described by Langenbach et al. [Proc. Natl. Acad. Sci. (U.S.A.), 1978, 75: 2864; and J. Natl. Cancer Inst., 1981, 66: 913]. Cytotoxicity was shown by a reduction in colony size at 1 ppm (ug vomitoxin/ml); by a reduction in the number and size of colonies at 2 to 3 ppm or higher; and by lethality to 80-90% of the cells at 10 ppm. Up to and including 3 ppm, vomitoxin was non-mutagenic to V79 cells at the hypoxanthine-guanine phosphoribosyl transferase (HGPT) locus, with or without hepatocyte-mediated activation; and did not increase the number of 6-thioguanine-resistant mutants at marginally cytotoxic levels of 6 and 8 ppm (data not shown). These findings suggest that vomitoxin, like other 13-epoxytricothecenes, may exert cytotoxicity through inhibition of protein and/or DNA synthesis, and is likely to be non-carcinogenic.
188. Pluronic, Polyols as Vehicles for Petroleum Derivatives in in vitro Assays. M. Harbois*, D. Marino, R. Papciak*, M. Grant, D. Crutchfield, and S. Brecher*, Gulf Life Sciences Center, Pittsburgh, PA 15238-2874

Pluronic® polyols (BASF Wyandotte Corp., Parsippany, N.J.) were tested for emulsifying properties, cytotoxicity, and effect on assay endpoint. F68 (10% in water) was found to be generally useful for dispersion of lighter fractions, while undiluted L121 and F127 (50% in ethanol) were effective with the heavier fractions. F68 was not toxic to Salmonella at 10 mg/plate, to CHO cultures at 0.04%, or to BALB/3T3 cells at 0.1% under test conditions. L121 (1%) was toxic to the BALB/3T3 cells after 3 days of incubation. CHO cells were not affected by F127, but were affected by L121 after 5 hours of incubation. In mutagenicity assays, F68 had no effect on the number of mutant colonies in Salmonella incubated with or without S9, or with S9 and 2-anthramine. Neither F68, L121, nor F127 had a significant effect on the number of mutant colonies in CHO cells incubated without S9, with S9 only, with ethylmethane sulfonate, or with benzalpyrene and S9. There was no increase over control in the number of transformed foci found in BALB/3T3 cells treated with F68, and no significant difference in the number of foci in cultures treated with 3-methylcholanthrene with and without F68. These results indicate that Pluronic® polyols can be used to disperse petroleum derivatives in aqueous media, and that selected polyols were neither toxic nor mutagenic for cells in culture.

189. The Influence of Metabolic Substrates Upon Hepatocyte Proliferation In Short Term Primary Cultures. J. A. McGowan* and N. L. R. Bucher*, Shriners Burn Institute and Surgical Services, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114

Adult rat hepatocytes, freshly isolated by collagenase perfusion and cultured for 3 days in an arginine and thymidine-free modification of Waymouth's MAB 87/3 medium are modestly stimulated to synthesize DNA by inclusion of either insulin (10^{-12} M) and epidermal growth factor (EGF, 10-100 ng/ml), or dialyzed normal rat serum (NRS, 5-30%). This stimulation is substantially influenced by introduction of various metabolic substrates. In E+EGF stimulated cultures, in the complete absence of glutamine or any carbohydrate energy source, addition of glucose (5.5-41 mM) enhances DNA synthesis (5'H-thymidine incorporation) by 30-100%, 5.5 mM being optimal; pyruvate magnifies DNA synthesis 4-fold, whereas lactate by itself is only half as effective, but approaches the potency of pyruvate when combined with glucose (5.5-27 mM). In contrast, glutamine (0.4-10 mM) is inhibitory, but is opposed by pyruvate. In NRS-stimulated cultures the effects are similar. Mitotic indices of up to 15-20% were observed under optimal conditions in sparsely populated areas of 17 hr colchicine blocked cultures. It is clear that even with high concentrations of EGF or NRS, maximum proliferative rates are not attained unless large amounts of pyruvate or lactate are present. Glucose, though sometimes stimulatory, is not necessary for maximal activation, and glutamine (a component of most culture media), is inhibitory. (Supported by USPHS grants CA02146 and AM19435 and a grant from the Shriners Hospitals for Crippled Children.)

190. Modulation by Vitamin A Acetate of the Mitogenic Activity of EGF and Insulin on Prostatic Epithelium in Defined Medium. D. M. Chaproniere-Rickenberg* & M. M. Webber*, Div. of Urology, Box C-319, University of Colorado Health Sciences Center, 4200 E. Ninth Ave., Denver, CO 80262

With the advent of simple defined media for the growth of epithelial cells it has become possible to study not only those factors increasing cell proliferation (mitogens) but also regulators of cell proliferation. Prostatic acini cultured in RPMI-1640 basal medium are stimulated to proliferate by insulin and EGF. Dexamethasone and retinoic acid do not affect proliferation in RPMI-1640 except in the presence of insulin or EGF, when they alter the response of the cells to the mitogen. Retinol acetate at concentrations of less than 10^{-3} I. U./ml inhibits the mitogenic activity of EGF but not of insulin. At higher concentrations retinol acetate increases the mitogenicity of both EGF and insulin, with an optimum at 10^{-1} I. U./ml retinol acetate. The action of retinol acetate thus parallels that of dexamethasone, differing only in that low concentrations (10^{-11} M) of dexamethasone inhibit insulin activity while retinol acetate does not. The effects of retinol acetate and dexamethasone appear to be additive. The implication of these results in growth regulation of normal and neoplastic cells is of interest. (Supported by DIHS, NCI, Division of Cancer Cause and Prevention Grant CA 28279 to Webber.)

191. Influences of Culture Media on the Survival and Neuritic Growth of Chick Embryo Ciliary Ganglion Neurons in Monolayer Culture. C. Hewitt*, S. M. Matthes*, S. D. Skaper, G. E. Davis and S. Varon, Dept. Biology, M-001, Univ. Calif. San Diego, La Jolla, CA 92093

Neurons in monolayer culture require distinct protein agents for survival (neurotrophic factors) and neuritic growth (neurite-promoting factors). However, neuronal survival and growth may not be maximally expressed if the culture medium is nutritionally deficient or if additional media constituents are necessary for the action of the factors. Chick embryo ciliary ganglion neurons were cultured on polyornithine-coated plastic (PORN) in the presence of different basal media (BME, DME, MEM, F-12, L-15, RPMI-1640) and in the presence or absence of Ciliary Neurotrophic Factor (CNTF), PORN-binding neurite-promoting factor (PNPF), or serum. In our standard culture conditions, namely serum containing BME plus CNTF, more than 90% of the neurons seeded will survive over several days. Replacement of serum with the N1 supplement (PNAS, 1979, 76, 514) reduced neuronal survival to 40%. However, full survival could be restored if any of the other basal media were used. In all cases no survival occurred if CNTF was omitted. Neurite growth was expressed to some extent on PORN in all culture media and in all cases this growth was inhibited by serum. However, serum did not interfere with the known stimulation by PNPF of
neurite outgrowth. Under all culture conditions the proportion of neurons having neurites increased with time in vitro.

(Supported by NIH grant NS16349.)

192. Induction of Transglutaminase Activity in Transformed Human Cells by Sodium Butyrate.

P. J. BIRCKBICHLER*, G. R. Orr, M. K. Patterson Jr.*, E. Conway, M. Maxwell and P. J. A. Davies*, Biomedical Division, The Samuel Roberts Noble Foundation, Inc., P.O. Box 2180, Ardmore, OK 73402 and "Department of Pharmacology, University of Texas Medical School at Houston, Houston, TX 77025

Transglutaminase catalyzes calcium-dependent isopeptide formation between peptide-bound glutaminyl moieties and primary amines and is low in cells which have undergone viral transformation. The enzyme activity in WI-38 VA13A cells can be increased nearly 10-fold following exposure to sodium butyrate. The increase in activity is time and concentration dependent and reversible once sodium butyrate is removed. Studies with puromycin and actinomycin D showed that the increase in activity was the result of that synthesis of new enzyme. Examination of butyrate-treated WI-38 VA13A cells by the Western blot technique with affinity-purified cellular anti-transglutaminase antibody showed the increase of a polypeptide similar in molecular weight to that observed for transglutaminase in WI-38 cells. The affinity of the enzyme for the amine substrate putrescine was unaltered by the butyrate treatment. The butyrate-treated transformed cells also stained more abundantly for transglutaminase and pericellular fibronectin when examined by indirect immunofluorescence. These observations reaffirm a relationship between transglutaminase and pericellular fibronectin in transformed cells and offer a model system to study transglutaminase activity in transformed cells in culture.

193. Substrates of Cellular Transglutaminase Using WI 38 Cells Generate Isopeptide Bonds. M. K. PATTERSON, Jr.*, M. D. Maxwell, P. J. Birckbichler*, and E. Conway, The Samuel Roberts Noble Foundation, Inc., P.O. Box 2180, Ardmore, OK 73402

Our previous studies related transglutaminase, ε-(γ-glutamyl)lysine isopeptides, and polyamines to the proliferative state of the cell (Patterson, et al., Cell Biology's International Reports, 6: 461-470, 1982). Our model suggests that polyamines modulate isopeptide bonds and thus the proliferative state. Of particular interest are the endogenous substrates that participate in the protein-protein bond. Evidence that a high molecular weight protein, greater than 250 K is the amine acceptor while two proteins of approximately 250 and 220 K participate as the amine donors in the reaction. Antibodies prepared to the product were tested for cross reactivity to other proteins to establish their relationship.

194. Separation of Exponential Cultures of V-79 Cells by Counterflow Centrifugal Elutriation: Correlation of Protein and Superoxide Dismutase (SOD) Levels. E. D. WERTS, L. L. Laban, E. J. Petty, Department of Human Oncology, WCCC, University of Wisconsin, Madison, WI 53792. (Supported by M. N. Gould*.)

It is well known that cellular radiosensitivity varies with cell cycle position and that superoxide anions are one of several radiolytic products of cellular irradiation. We wished to determine whether SOD activity, which catalyzes dismutation of superoxide anions, changed during the cell cycle corresponding to variation in radiosensitivity. To that end, 24 hour cultures of V-79 cells in log phase growth in 700 cm² glass roller bottles were lightly trypsinized (0.1% trypsin for 2 minutes), collected in 20 ml of αMEM + 20% fetal bovine serum and sized with a Particle Data Celloscope. Then they were loaded into a Beckman J-E6 elutriator rotor to collect 16 fractions in phosphate buffered saline representing increasing increments of approximately 1 mm diameter difference. Enriched fractions containing G₀ cells appeared in fractions 2-4, S phase in fractions 8-11 and G₁/M in fractions 14-16 as measured by the fluorescence activated cell sorter and supported by tritiated thymidine uptake. SOD activity from trypsinized cultures or those scraped with a rubber policeman was not significantly different. We found a consistent increase in total cellular protein (Lowry assay) with increasing cell size and a concomitant increase in total SOD activity. However, there was no significant difference in total SOD activity per microgram protein between cells in fractions of small versus medium or large diameters. These data suggest that SOD activity does not change relative to cell cycle position. Supported by AMIDEAST Peace Fellowships and NIH Grant R25 CA18397.

195. Perspectives in Cyogerontology. L. HAYFLICK, Center for Gerontological Studies, 3357 GPA, University of Florida, Gainesville, FL 32611

The field of cell aging, or cyogerontology, is now about 25 years old. In that quarter decade the discipline has grown from skepticism about its validity to become a major contributor of fundamental knowledge about the aging process. Chief among the major findings in cyogerontology are (1) the inverse relationship of donor age to the population doubling capacity of that donors' cultured cells (2) the direct relationship between species life span and population doubling capacity (3) the universality of the mortality of cultured normal cells (4) the occurrence of several hundred incremental or decremental biological changes prior to the cessation of doubling capacity, many of which changes are identical to those characteristic of intact aging animals and (5) the attribution of the finite replicative capacity of cultured normal cells to genetic events. (Supported, in part, by Grant AG 00850 from the Founding Council of the National Institute on Aging, NIH)

196. Dominance of Finite vs. Infinite In Vitro Life-span in Somatic Cell Hybrids. J. R. SMITH* and O. Pereira-Smith, W. Alton Jones Cell Science Center, Lake Placid, NY 12946

We have recently found that somatic cell hybrids formed by the fusion of several different immortal cell lines with human diploid fibroblasts (HDF) have limited proliferative potentials similar to those of HDF's. These results
suggest that the phenotype of limited lifespan is dominated by such hybrids. We have also found that hybrid cells resulting from the fusion of immortal cell lines with other immortal lines exhibit either a finite division capacity or not depending on the immortal cell lines involved. Using an SV-40 transformed cell line as one of the parents in these cell hybridization experiments, we have identified two “complementation groups” within immortal cell lines. Fusion with other independently derived SV-40 transformed lines and HT1080 cells yielded immortal hybrids. However, when SV-40 transformed cells were fused with the cell lines HeLa, 143B or T98G the hybrids had a limited in vitro lifespan, ranging up to 70 PD. These results suggest that cellular immortality is the result of recessive changes in the genetic program responsible for limiting the proliferation of normal cells.

(This work was supported by NIH grants T32 AG00095 and AG03262.)

197. Senescent WI-38 Cells Bind Epidermal Growth Factor (EGF) but Fail to Express the EGF Receptor-Associated Protein Kinase Activity. C. Carlin, J. Golczewski, P. D. Phillips*, B. Knowles and V. J. CRISTOFALO*, The Wistar Institute, 36th Street at Spruce, Philadelphia, PA 19104

EGF acts as one of the principal mitogens for WI-38 cells. As cultures of these cells senesce there is a progressive decline in the number of cells responding to EG\(\text{F} \) by initiating DNA synthesis. Loss of responsiveness is not due to a loss in the ability of senescent cells to bind EGF. In fact there is an actual increase in the number of receptors per cell. Using an antisera specific for the human EGF receptor we immunoprecipitated receptors from radiiodinated and inorganic \(^{32}\text{P}\)-labeled young and old cells and were not able to detect any differences in their electrophoretic properties after 2-dimensional gel electrophoresis. However, receptors isolated from young cells display the tyrosine-specific ATP-dependent protein kinase activity associated with the EGF receptor while the receptors isolated from old cells do not display this activity. Although the role of the EGF receptor-associated kinase activity in the mitogenic response is unclear, we believe that this finding represents the first mitogen-specific alteration associated with senescent cells. (Supported by NIH Grants AG-02815, AG-00378 and CA-18470 and CA-10815).

198. The Role of Protein and RNA Synthesis in the Inhibition of Nuclear DNA Synthesis in Heterokaryons Resulting from the Fusion of Senescent and Low Passage, Actively Dividing Human Diploid Fibroblast-like Cells. T. H. NORWOOD, G. C. Burmer and C. J. Zeiger, Department of Pathology, University of Washington, Seattle, WA 98195 (Sponsored by L. Hayllick)

Previous studies have demonstrated dominance of the senescent phenotype with respect to nuclear DNA synthesis in heterokaryons resulting from the fusion of late passage, post mitotic (senescent) and early passage, actively replicating human fibroblast-like cells. More recently we have shown that brief post-fusion treatment with the protein synthesis inhibitor cycloheximide or puromycin delays the inhibition of entry into DNA synthesis of young nuclei in heterokaryons with the senescent cells. In contrast, prefusion (but not post-fusion) treatment with the reversible RNA synthesis inhibitor 5-6-Diethyl-1-B-D-ribosulfanimidazol (DRB) results in a significant increase in the percent of \(^{3}\text{H}\) thymidine labeled nuclei in old-young heterokaryons immediately following fusion. Based on these results and the observation that the DNA synthetic inhibitory activity can be transferred via enucleate cytoplasts from senescent cells, we, and other investigators, have proposed that an inhibitor of DNA synthesis, dependent upon protein and RNA synthesis, is present in senescent human fibroblast-like cells.

199. Amplification of an “Inter-Alu” Sequence in Extrachromosomal DNA Circles During In Vitro Aging of Normal Human Cells. R. J. SHMOOKLER REIS, C. K. Lumpkin, Jr., J. R. McGill, K. T. Riabowol and S. Goldstein*, University of Arkansas for Medical Sciences and VA Medical Center, Little Rock, AR 72205

We have utilized a moderately repetitive DNA sequence (2-30 copies/cell) to monitor DNA rearrangements near the highly repetitive Alu repeats (>600,000 copies/cell) with which they are associated in the human genome (Calabretta et al., Nature 296: 219-225, 1982). We examined DNA from serially passaged diploid human fibroblasts, in which we had previously demonstrated progressive loss of reiterated sequences (Cell 21: 739-749, 1980). Four of six strains examined showed amplification at late passage. Of extrachromosomal DNA hybridizing to the Inter-Alu probe (by 2-5-fold) to >8-fold while chromosomally integrated copies remained constant. Sizes of amplified Inter-Alu DNA, after Bam HI cleavage, ranged from 1.6 to 8 kb, with 1 to 3 bands apparent in each strain. The amplified copies were shown to be extrachromosomal, and to include covalently closed circles. A single size class (4.8 kb) of extrachromosomal DNA containing Inter-Alu was also amplified in normal lymphocytes, predominantly in B cells, isolated from 16 of 24 old donors (61-91 yr) but not from any of 18 young donors (21-31 yr). Both in vitro and in vivo aging are thus associated with the amplification of Inter-Alu circles. Such extrachromosomal DNA elements may contribute to senescent decline or may reflect a more general loss of genomic stability during the lifespan.

200. EGF Stimulated Cell Proliferation and Proliferation-Associated Protein Synthesis in Young and Old WI-38 Cells. P. D. PHILLIPS* and E. J. Kuhnle, The Wistar Institute, 36th at Spruce Street, Philadelphia, PA 19104

Density arrested cultures of young WI-38 cells can be stimulated to proliferate by refeeding with serum supplemented medium or with serum-free MCDB-104 supplemented with epidermal growth factor (EGF), insulin, transferrin and dexamethasone (4 factor medium). Old cultures of WI-38 cells respond poorly to refeeding with either serum supplemented or 4 factor medium. In 4 factor medium EGF acts as a principal mitogenic signal. We have previously determined that EGF-receptor binding appears essentially the same in young and old cells, and thus, it is not a simple loss of binding that prevents a
proliferative response. We have now begun to examine the synthesis of putative proliferation-associated proteins. Density arrested young and old cells are refed with 4 factor medium and pulsed with \(^{3}P\)-Met for 1 hours periods until the entry of cells into DNA synthesis (12-15 hours). Cytosol extracts are subjected to nonequilibrium pH gradient electrophoresis (NEPHGE) followed in the second dimension SDS-PAGE and then processed for autoradiography. Analysis has revealed at least 1 protein which is preferentially synthesized by cultures of young cells within the first 2 hours following the application of the mitogenic stimuli. This protein migrates near the center of the NEPHGE gels and in the second dimension has an apparent molecular weight somewhat greater than 100,000 daltons. Supported by NIH Grants AG-02851 and AG-00378.

201. The Effects of Glucocorticoid Conditioned Medium upon the Proliferation of WI-38 Cells. C. FINLAY and V. J. Cristofalo*, The Wistar Institute, 36th at Spruce Street, Philadelphia, PA 19104

Chronic exposure to glucocorticoids results in a 25-30% increase in the proliferative life span of WI-38 cultures. We have previously reported that the stimulatory effects of hydrocortisone (HC) or dexamethasone (DEX) upon the proliferation of WI-38 cells are mediated by a glucocorticoid-induced conditioning of the medium following subcultivation (HC-CM and DEX-CM, respectively). Although the responsive period of WI-38 cultures to the hormone is limited, the proliferation of both low density and confluent quiescent cultures is increased in response to HC-CM. Cultures refed during every growth cycle with HC-CM demonstrate a 25% increase in their proliferative life span when compared to cultures refed with control conditioned medium (CM) or CM plus HC. The action of the putative factor is not dependent upon the presence of the hormone; cultures seeded into or refed with medium dialyzed to remove stimulatory levels of DEX achieve saturation densities 30-40% higher than controls. Both fetal lung cell lines stimulated by glucocorticoids and fetal skin cell lines inhibited by glucocorticoids generate HC-CM stimulatory to WI-38 cultures. This activity, which is dialyzable (12,000 M.Wt. cut-off tubing), may be recovered in the less than 10,000 M.Wt. fraction following ultrafiltration. Experiments are being conducted to further characterize this factor(s) and its effects upon the proliferation of other cell lines. Supported by NIH Grant AG-00378 and GM-07229.

202. The Presence of non-Cycling Cells at All Levels of Population Doubling of IMR-90 Fibroblasts. L. N. CASTOR,* Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111

The role played by production of non-cycling cells in progression of IMR-90 cells toward senescence was investigated using two departures from conventional methods: 1) The cells were plated at a low population density \((20/mm^{2})\) and kept in rapid growth by frequent feeding and subcultivation while non-confluent. 2) For measurement of the labeling index, \(^{3}H\)-thymidine was added for 6-7 days at a concentration \((1 \mu Ci/ml)\) that inhibited division of labeled cells. The percentage of unlabeled cells increased exponentially with population doubling level (PDL), from 5% at PDL 15 to 82% at PDL 65. Cells plated after mitotic selection at PDL 26 had a near-Gaussian distribution of rates of passage through the G1 phase (G1 rates), with a finite proportion of non-cycling cells indicated by extrapolation of the distribution to a G1 rate of zero. Clones initiated after mitotic selection had varying proportions of unlabeled nuclei. The data suggest that non-cycling cells appear in all cell lineages, at all levels of population doubling, and that they result from the wide variation in the rates at which individual cells are capable of completing the G1 phase. Cessation of culture growth ("phase III") results when non-cycling cells are produced more frequently than cycling cells, without a sudden change in cycle characteristics. (Supported by NIH grants CA-06927, CA-22780, and CA-24961, and by an appropriation from the Commonwealth of Pennsylvania.)

203. Reorganization of the Genome During Aging of Human Fibroblasts. A. MACIEIRA-COELHO* and F. Puvion-Dutilleul, Institut de Cancérologie et d’Immunogénétique (INSERM) and Institut de Recherches Scientifiques sur le Cancer (CNRS), 94804 Villejuif, France

We have previously suggested that aging of human fibroblasts is the result of the genome reorganization taking place during cell division. This hypothesis was checked following the partition of DNA during the division cycle and the organization of chromatin during serial population doublings (PD) of embryonic and postnatal fibroblasts from normal donors of different ages and from donors with Werner’s syndrome. Cytophotometry after ethidium bromide and Feulgen-pararosaniline staining showed that at each PD in 20% of the cells DNA is unequally distributed between sister cells. Grain counts after \(^{3}H\)-Tdr labeling and autoradiography showed that newly synthesized DNA is unequally distributed. Fluorescent-Giemsa staining after BrdU incorporation showed a high frequency of asymmetric DNA synthesis. An accumulation of thermo- and alkali-labile sites during serial subcultivation of fibroblast populations was found with alkaline sucrose gradients. Analysis of chromatin at the ultrastructural level after Miller’s spreads suggested that the fragile sites are located at the nucleosomal level. Part of the DNA of old cells appeared fragmented in a linear or circular form. The results fit the hypothesis that transposable elements which normally detach from chromosomes during the division cycle fail to reintegrate in old cells due to changes in chromatin structure.

204. Trauma as a Means of Initiating Change in Genome Organization and Expression. B. MCCINTOCK, Carnegie Institution of Washington, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

Responses of genomes to shock may be programmed or they may be improvised and result in various types of genome modification. Cells of maize in which one arm of one chromosome is continuously restructured over a limited number of successive mitotic cycles respond by activating potentially transposable elements that previously could not be identified as such. This sensing occurs almost immediately as evidenced by activations detected as early as the second mitotic division following
initiation of the cycle. Insertion of such an element at a gene locus may start a sequence of altered types and patterns of gene expression. Besides such activations the trauma may initiate major chromosomal reorganisations. Evidence from various sources supports the notion that trauma ("genome shock") may be effective in providing a wide range of new and unanticipated organisations and expressions of a genome.

205. Characterization of Putative Transposons in Cytoplasmic Male Sterility of Maize. R. J. Mans, Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL 32610

Maternal inheritance of male sterility, subject to restoration to fertility by a single nuclear allele, makes the maize oiler a facile entree into the molecular genetics of fertility. Recently, we have used cloned segments of S-1 nuclear sequences homologous to S-1 and S-2 and have spontaneously reverted to fertility with cloned segments of S-1 and S-2 revealed reorganization of the genome suggestive of transposition. We have sought and found nuclear sequences homologous to S-1 and S-2 and have correlated their presence with the propensity of certain nuclear backgrounds toward reversion of sterile lines to fertility. Recently, we have used cloned segments of S-1 and S-2 as templates for in vitro transcription with an a-amanitin sensitive RNA polymerase that transcribes the inserted maize segments preferentially. We are now seeking altered transcription of S-1 and S-2 sequences coincident with rearrangement of the mtDNA. The phenomenon of cytoplasmic male sterility, restoration to fertility by nuclear genes and reversion to fertility by genomic rearrangement may be a manifestation of controlling elements extending beyond the nuclear genome.

206. Genetic Manipulation of Chloroplasts and Mitochondria by Plant Protoplast Fusion. L. Menczel, F. Nagy, P. Medgyesy, P. Maiga, Institute of Plant Physiology, Biological Research Center, Szeged, Hungary H-6701

Important traits coded by chloroplast and mitochondrial DNA (e.g. herbicide resistance and cytoplasmic male sterility) are difficult to manipulate by conventional breeding techniques since these organelles are inherited in a uniparental-maternal way in most higher plants. With protoplast fusion there is no specific mechanism to eliminate one or the other type of parental genome, therefore "inheritance" is biparental. In progeny cells derived from protoplast fusion products, the parental nuclei and organelles segregate independently resulting, in some cases, in new nucleus/chloroplast/mitochondrion combinations.

The transfer of chloroplasts and mitochondria by protoplast fusion between different Nicotiana species will be demonstrated, and the efficiency of different selection systems applied in organelles transfer experiments will be discussed.

207. In Vitro Plant Transformation by Bacterial Co-Cultivation and Expression of Foreign Genes in Plant Cells. R. T. Fraley, S. G. Rogers and R. B. Horsch, Monsanto Company, Corporate Research Laboratories, St. Louis, MO 63167

Chimeric bacterial genes conferring resistance to amino-glycoside antibiotics have been inserted into the Agrobacterium tumefaciens Ti plasmid and introduced into plant cells by in vitro transformation techniques. The chimeric genes contain the nopaline synthase 5' and 3' regulatory regions joined to either neomycin phosphotransferase Type I or Type II. The chimeric genes were cloned into a novel intermediate vector, pMON120, and inserted into pTiB653 by co-integration and then introduced into petunia and tobacco cells by co-cultivating A. tumefaciens cells with protoplast derived cells. Southern hybridization was used to confirm the presence of the chimeric genes in the transformed plant tissues. Expression of the chimeric genes was determined by the ability of the transformed cells to proliferate on medium containing normal inhibitory levels of kanamycin (50 µg/ml) or other aminoglycoside antibiotics. Plant cells transformed by wild type pTiB653 or derivative carrying the bacterial neomycin phosphotransferase genes with their own promoters failed to grow under these conditions.

208. New Developments in the Transformation of Plant Tissues. M. P. Gordon, E. Nester, F. White, R. Amasino, A. Powell, B. Taylor, G. Huffman, G. H. An, T. Nagata (Sponsored by K. Redenbaugh), Departments of Biochemistry and Microbiology and Immunology, University of Washington, Seattle, WA 98195

Plants can be regenerated from tumor tissues generated on Nicotiana tabacum Wisconsin 38 by Agrobacterium tumefaciens B6. These plants have relatively normal morphology. They were fertile and contain DNA derived from the tumor inciting plasmid. Plant tissues transformed by Agrobacterium rhizogenes form tumors from which roots develop. These roots, in turn, form fertile plants. Some, if not all, of the plasmid DNA is retained throughout these changes. The Agrobacterium rhizogenes system is distinguished by homology between the bacterial root-inciting plasmid and many plants. This homology may be significant in terms of the origin of the transforming plasmids of Agrobacteria. Transient expression of foreign genes in protoplasts has been obtained.

(Supported in part by National Cancer Institute grant, American Cancer Society grants, and Standard Oil Company (Indiana).

209. Insights into Human Cancer Using Organ Cultures. J. C. Petricciani*, I. Levenbook*, Office of Biologies, NCDB, FDA, 8800 Rockville Pike, Bethesda, MD 20205

Because cancer is a disease in which there is proliferation and invasion of abnormal cells into normal tissues, we have used normal human muscle (HM) in organ culture as a substrate to study the proliferation and invasion of human tumor cell lines as well as primary human tumor cells. Our results show that the histopathology of the HM system mimics that seen in vivo in humans and in experimental animals. A comparison of several human tumor cell lines (e.g., HeLa, WiDr, HT-1080, and others) shows that the HM system can differentiate the
invasive potentials of those lines. In addition, the HM system has been used to identify subpopulations of cells with differing biologic behaviors. The HM system provides a rapid and sensitive method for evaluating the transformation of cells by chemical carcinogens and oncogenes.

210. Migration of a Rat Bladder Carcinoma Cell Line on Solid Substrata and in Three-Dimensional Tissue. R. TCHAO, W. Schroyens, and J. Leighton.* Department of Pathology, Medical College of Pennsylvania, Philadelphia, PA 19129

We have been investigating whether or not the motility of carcinoma cells is related to their invasiveness. NBT-II, a rat bladder carcinoma cell line, exhibits a rotatory movement of cells in groups on glass or plastic. On a film of type I collagen these cells move independently of each other. In a three-dimensional system using embryonic chick heart fragments, NBT-II cells migrate readily into the tissue. This in vitro system to study cell invasion has been correlated with the invasiveness of tumor cells in vivo. In the presence of succinyl Con-A (s-ConA), cell motility on glass or collagen ceases at once, and the migration of cells from an aggregate on to a solid substratum is inhibited by 80%. However, in the chick heart model, in the presence of s-ConA, NBT-II cells continue to infiltrate inside the tissue. Our results indicate that the mode of cell motility on different solid substrata may differ qualitatively, as well as quantitatively. Furthermore, the control of cell motility in two-dimensional and three-dimensional cultures may be different. (Supported by NCI Grant CA 14137).

211. Anchorage Independent Survival as an Indicator of Malignant Transformation: Morphological and Biochemical Comparison. W. A. SUK*, J. E. Humphreys, and E. P. Hays, Northrop Services Inc., Environmental Sciences Division, Research Triangle Park, NC 27709

Murine leukemia virus-infected rat cells have been used in an assay in which enhanced anchorage independent (AI) survival is used as an indicator of neoplastic transformation. Modification of the assay to include a 3 day replicative phase between a 3 day carcinogen treatment and 4 day agar phase yielded dose-responses with 5 known carcinogens. Morphological comparisons of control and carcinogen-treated cells during agar phase were made. Untreated and solvent control cells are spherical in shape and are uniformly planar in suspension. Carcinogen-treated cells formed multicellular aggregates suspended in three-dimensional arrays; these cells vary in shape and exhibit an increase in cell size, number, and intracellular material, as well as a thickening of the cell wall. AI cells show a reduction in RNA, DNA, and protein synthesis; RNA and DNA synthesis is further reduced in carcinogen-enhanced AI cells. In an attempt to understand these morphological changes, the nuclear proteins of AI cells were analyzed by polyacrylamide gel electrophoresis. AI cells showed the disappearance of a major protein of 45K dalton molecular weight (MW), a shift to proteins of lower MW, and differences in band patterns between treated and untreated cells. The data provide a system for the detection of carcinogen-induced changes in virus-infected rat cells and for the study of morphology-related gene expression. (NIH/NTP Contract #N01-ES-15798)

212. Phenotypic Variability in Expression of Anchorage Independence by a Human Breast Tumor Cell Line. M. C. Hancock,* A. J. HACKETT,* and H. S. Smith,* Pennta Cancer Research Institute, Oakland, CA 94609

Possible mechanisms responsible for anchorage independence were investigated using a human breast carcinoma cell line, Hs578T. We found that expression of anchorage independence was not always stable, and most likely occurred via alterations in gene expression, which induced secretion of specific growth factor(s). The colony forming efficiency in methocel increased with higher initial plating density and drifted up with passage in culture. At high passages, growth in methocel was less sensitive to initial plating density. Clonal variants isolated in methylcellulose maintained a higher plating efficiency when kept in suspension, and removal of selective pressure resulted in the loss of the high level of expression upon subsequent challenge in suspension. Clones requiring anchorage acquired the ability to grow in methylcellulose after only one passage in culture; such a rapid rate of variation is not typical of somatic mutation and suggested alternate mechanisms. Exposure to 1 µg/ml of 5-aza-cytidine, which decreases methylation of DNA and may thereby activate gene transcription, increased the incidence of anchorage independent growth 20 times; whereas, treatment with 6-aza-cytidine had no effect. Unconcentrated medium conditioned by cells previously exposed to 5-aza-cytidine or from high passage cells stimulated growth in suspension by as much as 10.9 times. The secreted factor(s) was stable to heat up to 65°C for 30 minutes and was larger than 12,000-14,000 daltons as determined by dialysis.

213. The Analysis of Some Interactive Processes in Mammary Disease. H. L. HOSICK,* C. A. Carrington, N. Choongkittaworn, Department of Zoology, Washington State University, Pullman, WA 99164.

Tissue-level organization aids in maintenance of differentiated function (e.g., lactose and fatty acid synthesis) of mammary cells in culture. Tissue-level events appear also to play a role in mouse mammary tumor development and we are now using collagen matrix cultures to analyse these in detail. We have measured several characteristic functions of mammary epithelium in collagen gel, comparing dispersed cells and multicellular units of several sorts (ducts, end-buds, cell spheroids), from normal and neoplastic glands. Patterns of growth and morphogenesis of the various multicellular units examined are distinctly different. Outgrowths from ducts and from end-buds/alveoli of the normal gland, and from multicellular tumor spheroids are all distinguishable in this culture system. Growth pattern and growth rate of tumor spheroids are different from those of tumor cell dispersions, are modified by spheroid seeding density, and are dissimilar in different tumorigenic cell lines. These differences reflect some of the behavioral characteristics of the lines in vivo. Our findings indicate the importance of tissue-level organization.
in mammary pathobiology. The three-dimensional collagen matrix culture system provides a useful approach for detailed consideration of how such organization contributes to the expression of a variety of cell properties of importance in normal and neoplastic mammary development. (Supported by NIH grant AG-02909).

214. Epithelium from Human Breast Cancers in Culture: Is it Really Cancer? R. C. HALLOWES*, L. A. Peachey, S. Cox, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX
Techniques have been developed during the past 5 years that enable primary cultures of normal and dysplastic human breast epithelium to be readily and routinely acquired to digest tissue. They have difficulty attaching to reversibly during tissue dissociation by the proteases required to digest tissue. They have difficulty attaching to and spreading upon native type I collagen gels. It may be weeks rather than hours before cancer cell clusters adapt and grow on or in their new environment and the method of growth is different from that observed for non-cancer cells. Once these differences are recognized it becomes easier both technically and conceptually to routinely initiate primary cultures of breast cancer and to examine the effects of cells, substrate and media composition upon their growth and differentiation. Such studies indicate that the provision of a suitable extracellular matrix upon which the cancer cells can spread is of the greatest importance.

215. Experimental Surgical Pathology Using Histophysiologic Gradient Culture. Stimulation and Analysis of Organoid Association of Cells. J. LEIGHTON,* R. Tchao, and R. F. Nicosia, Department of Pathology, Medical College of Pennsylvania, Philadelphia, PA 19129
Cancer is diagnosed by finding characteristic structural abnormalities in a tissue. The spatial requirements for simulating in culture such qualities include three-dimensional growth in confined spaces enabling cells to form complex groups under conditions where the gradients of diffusion can be recognized. These conditions are provided in histophysiologic gradient culture. We have compared polarization and proliferation of normal and neoplastic rat urothelium (NBT II), and also the interaction between urothelium either normal or cancerous, and microvessels emerging from rat aorta. NBT II, cultured on a membrane of permeable collagen, forms a stratified epithelium. Which surface of this epithelium proliferates is determined by the side of the epithelium engaged in metabolic exchange with the medium. Normal bladder grows as stratified hyperplastic epithelium, proliferation occurs only on the interface with the supporting collagen membrane, whether nutrients are received across the collagen membrane or on the free surface of the epithelium. When branching microvessels contact NBT II, it proliferates and spreads rapidly on the vascular structures. Normal urothelium in the same setting does not. Differential qualities of polarization and response to contact with branching microvessels may serve as indices in the characterization of cells as cancer. (Supported by NCI Grant CA 14137).

216. Fish Cell Line: Persistent Infection with a Coronavirus. B. LIDGERDING,* U.S. Fish and Wildlife Service, National Fish Health Research Laboratory, Kearneysville, WV 25430 and F. Hetrick,* University of Maryland, College Park, MD 20740
A putative coronavirus has been detected in an established, widely used, cell line derived from an epithelioma of common carp (EPC). The presence of syncytia in cultures incubated at 30°C suggested possible viral latency. Virtually no syncytia were seen in cultures grown at 15 and 20°C but as the incubation temperature was elevated the syncytia became more numerous. Viable cultures can be maintained at 30 to 35°C despite numerous syncytia if cultures are frequently subcultivated. The EPC cultures were determined to be free of microbial contaminants. Electron micrographs of cell lysates revealed 90-100 nm particles with morphologies characteristic of coronaviruses. The particles banded at a density of 1.18-1.20 in sucrose gradients, also typical of coronaviruses. No destructive cytopathology was evident in brown bullhead (BB), bluegill (BF-2), goldfish (CAR), chinook salmon (CHSE-214), fathead minnow (FHM), or rainbow trout (RTG-2) cell lines following inoculation of culture fluids or cell lysates. Destructive cytopathology was evident in FHM cultures treated with 5-iododeoxyuridine, other lines remained nonsusceptible. Culture fluids diluted beyond 1:100 produced no cytopathology. Origin of the virus remains unknown, however, syncytia were seen in EPC cultures obtained from United States and European laboratories and cultivated at elevated temperatures. This is the first report of replication of a coronavirus in fish cells.

217. Differential Interferon Sensitivities of Lethal and Non-lethal Strains of Rift Valley Fever Virus (RVFV) In Vitro. J. A. ROSEBROCK*, G. W. Anderson, Jr., H. Schellekens and C. J. Peters, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD 21701, and The Primate Center, TNO, Rijswijk, The Netherlands
A significant difference in lethality was observed when Egyptian (ZHS01) and South African (SA75) strains of RVFV were inoculated into Wistar-Furth (WF/HsdBR) rats (log LD50 equal to 0.7 and 6.7 plaque forming units, respectively). Since the interferon sensitivities of these two closely related strains of RVFV could be a critical factor in determining lethality, we initiated a series of in vitro experiments to compare the interferon sensitivity of ZHS01 and SA75 in fibroblast cultures derived from WF rats. Untreated cultures of WF fibroblasts replicated ZHS01 and SA75 equally well. Differences in interferon sensitivity were rapidly evident, however, if the cultures were pretreated for 20 hr prior to virus challenge with decimonal dilutions (0.1-1000 U/ml) of partially purified rat interferon—β(IF). A significant decrease in SA75 yield (1 log) was observed at the lowest IF dosage (0.1 U/ml) and replication was completely inhibited at
100 U/ml. To effect a 1 log reduction in ZH501 yield, 100 U/ml were required. Increasing the IF concentration to 1000 U/ml caused a greater reduction, but did not completely inhibit ZH501 replication. Hence, compared to SATV, ZH501 appears to have a diminished IF sensitivity in WF fibroblasts. If this is a characteristic of other WF cell types as well, it could contribute to the rapid death of these rats following in vivo inoculation of ZH501.

218. Cellular Studies Involving Bovine Leukemia Virus (BLV). G. F. AMBORSKI*, J. Lo, D. Samid, G. Richardson and J. Casey, Dept. Veterinary Science, Veterinary Microbiology and Parasitology, Veterinary Clinical Sciences, Louisiana State University, Baton Rouge, LA 70803 and Dept. Biochemistry, LSU Medical Center, New Orleans, LA

Bovine lymphocytes, tumors, sperm cells and permissive and non-permissive cell lines were examined for cellular characteristics of BLV. The presence of BLV provirus in cellular DNA was examined with a cloned BLV probe. The standard DNA extraction techniques were used for most of the cells but modifications were developed for the routine extraction of DNA from bovine semen. The production of BLV was measured by reverse transcriptase production and agar gel immunodiffusion tests. Transformation of the cell lines was examined by the demonstration of growth in soft agar. Lymphocytes and tumors from BLV positive animals as well as several permissive and non-permissive cell lines showed BLV provirus in the genome. Provirus has not been detected to date in DNA from sperm obtained from BLV positive or negative animals.

219. Enhanced Expression of EBV Viral Capsid Antigen Associated with Benzene Induced Architectural Changes in the B95-8 Line. E. V. Orsi,* S. L. MAINES,* G. Ragona* and S. Resta, Biology Dept., Seton Hall Univ., So. Orange, NJ 07079, and Clinica Medica Gen. III., Universita di Roma 00161, Roma, Italy

EBV-B95-8 virus (EBV) infected marmoset cells were exposed to levels of benzene which decreased lysosome vital dye retention and enhanced herpessimplex release from human diploid cells. The percentage of cells showing VCA by indirect immunofluorescence (FA) was measured at 1, 3, 5 and 7 days of passage. Staining increases in positive cells at 1 and 3 days were seen with benzene. At 5 days controls had 25% fewer positive cells but by 7 days no differences were seen. Intracellular and nuclear FA staining was distinctly brighter with benzene but cytoplasmic antigen localization resembled that of controls. Benzene also enhanced loss of acidine orange from lysosomes following in vitro UV irradiation shown by a 50% drop from control retention by photometric analysis. Benzene induced lysosome fragility was accompanied by pronounced loss of fibers linking the larger stress fibers of the cytoskeleton seen with Coomasie blue R 250. However, there were no differences in transformation efficiency of normal human lymphocytes after exposure to benzene during infection with EBV regardless of donor age or experience with infectious mononucleosis.

220. Semiquantitative Morphologic Analysis to Quantify the Cytotoxicity of Cadmium to Cultured Hepatocytes. E. M. B. SORENSEN and D. Acosta*, Department of Pharmacology/Toxicology, The University of Texas, Austin, TX 78712

Quantification of cytotoxic responses has been consistently problematic in monitoring changes which occur in populations of cells exposed to xenobiotics. In this study cultured parenchymal hepatocytes from rats were exposed to variable doses of cadmium, prepared for electron microscopy, and scored for the degree of morphologic change. One-day old cultures were exposed to 10-200 µM cadmium chloride for 15 minutes to 4 hours prior to fixation in glutaraldehyde and preservation for optical, scanning and transmission electron microscopy. Extracellularly, changes included reductions in the numbers of microvilli, peripheral blebbing, shape changes, swelling and surface irregularities such as the presence of enormous cavernous regions following the most severe treatments. Intracellularly, cells were characterized by peripheral vacuolization, nuclear pyknocytosis and pleomorphism, reductions in rough endoplasmic reticulum and smooth endoplasmic reticulum proliferation. Micrographs of treated and untreated parenchymal hepatocytes were scored according to an arbitrary scale from 0 to 4 in order of increasing severity of abnormalities to correlate cadmium-induced morphological changes with the severity of the exposure to cadmium. These data showed that as cadmium concentration and time of exposure were increased, the percentage of affected cells increased.

221. Cardiotoxicity of Tricyclic Antidepressants in Cultured Rat Myocytes. D. ACOSTA* and K. Ramos*, Department of Pharmacology and Toxicology, College of Pharmacy, University of Texas, Austin, TX 78712

The effects of amitriptyline, desipramine, imipramine and nortriptyline were studied in primary cultures of rat myocardial cells. Lactate dehydrogenase (LDH) leakage, cellular viability and beating rates were evaluated to compare their cardiotoxicity. Tricyclic antidepressants (TCAs) were added to the cultures to give a final concentration of 1 × 10⁻⁵, 1 × 10⁻⁴ and 1 × 10⁻³ M. Treatments lasted 1 and 4 hr. All TCAs tested caused significant release of LDH and decreased cellular viability when exposed to 1 × 10⁻³ M for 1 and 4 hr. Amitriptyline was the only compound that caused significant LDH release 4 hr after exposure to lower doses. Decreased viability was observed 4 hr after exposure to all TCAs at a concentration of 1 × 10⁻⁴ and 1 × 10⁻³ M. Arrhythmias were observed 1 hr after exposure to 1 × 10⁻⁴ and 1 × 10⁻³ M amitriptyline. All doses of amitriptyline inhibited beating 4 hr after exposure. Imipramine, desipramine and nortriptyline at a concentration of 1 × 10⁻³ M decreased the beating rates of cultured myocytes 1 and 4 hr after exposure. Arrhythmias and/or total inhibition of beating were observed when the cultures were exposed to higher concentrations of these compounds. Based on these data, the rank order of cardiotoxicity was: amitriptyline < desipramine < nortriptyline. (Supported in part by the University of Texas Research Institute).

222. Good Correlation between the Cytotoxicity of Drugs and their Allergic Side Effects. B. R. Ekwall,* Toxicology Laboratory, National Food Administration, Box 622, S-75126 Uppsala, Sweden
To investigate whether tissue culture methods can be used to predict the allergenic potential in man of chemicals, the cytotoxicity of the therapeutic blood levels of 81 drugs was compared with reports in the literature on allergic side effects. The cytotoxicity of the drugs was calculated as the ratio of the therapeutic, steady-state plasma concentration to the previously determined 50% inhibitory concentration for HeLa cells after 7 days' incubation in the MIT-24 test system, called CQTr (the cytotoxic quotient for repeated therapeutic dosage). CQTr values were then compared with reports on six categories of allergic side effects, i.e., 1. immediate allergic symptoms, 2. delayed allergic symptoms, 3. hemolysis, 4. drug-induced auto-immune disease, 5. drug-induced acute disseminated disease, and 6. drug-induced blood dyscrasias. Immediate allergy and blood dyscrasias were positively correlated with CQTr values, while remaining four categories were very well correlated with CQTr values. This might be due to an enhancement of the sensitization process by a drug-induced cell injury, which may stimulate lymphocytes and facilitate the binding of the drug hapten to cellular constituents. The auto-immune and acute disseminated diseases might be direct effects of drug cytotoxicity. Thus, tissue culture tests of cytotoxicity coupled to determinations of blood concentrations in man from exposure may predict sensitization potential of the chemicals.

### Table 223. Conditioned Medium Promotes Clonal Growth of Amniotic Fluid Cells. C. H. LAUNDON,* R. E. Priest and J. H. Priest,* Dept. of Pathology and Dept. of Pediatrics, Division of Medical Genetics, Emory Univ., Atlanta, GA 30322

| Medium                        | Colonies/Plate |
|-------------------------------|----------------|
| 1) complete D&V               | 6.8            |
| 2) 1/2 complete D&V + 1/2 AF-F| 24.2           |
| 3) complete D&V + 1mM pyruvate| 10.8           |
| 4) 1/2 complete D&V + 1/2 AF-F| 29.0           |
| conditioned + PIT             |                |
| Media 2 and 4 were both significantly better for clonal growth than 1 (p<.0001). The conditioned medium was then evaluated further. |
| 5) complete D&V               | 36.0           |
| 6) 1/2 complete D&V + 1/2 F   | 43.2           |
| conditioned                   |                |
| 7) 1/2 complete D&V + 1/2 AF  | 59.6           |
| conditioned                   |                |
| 8) 1/2 complete D&V + 1/2 AF-F| 49.0           |
| conditioned + PIT             |                |

Compared to 5, 7 gave the best colony growth (p<.0001). 8 was also significantly better than 5 (p<.025). Conditioned media is inexpensive and effective for promoting colony growth of AF and F cells.

224. Interleukin-1 Promotes Fibroblast Proliferation in Monolayer, Not in Soft Agar, and Not Mediated Through the EGF-Receptor. L. M. Mulhall, S. R. Newcom,* C. W. Bitter and M. S. Borzy, Oregon Health Sciences University, Portland, OR 97201

Interleukin-1 (IL-1) is a monokine which stimulates thymocyte proliferation and the production of IL-2. We measured the growth of NRK-49F fibroblasts and human dermal fibroblasts in monolayer in response to 10 μl of highly purified human IL-1 added to 35-mm dishes every day. We compared the proliferation to that achieved with Fibroblast Growth Factor (FGF) 100 ng/ml added every other day and to that achieved with weekly media changes only. On day 9 NRK-49F fibroblasts showed a 102 fold increase with IL-1, a 63 fold increase with DMEM and a 71 fold increase with FGF. When human fibroblasts were used IL-1 stimulated a 72 fold increase as compared to a 53 fold increase with medium alone and a 57 fold increase with FGF. IL-1 does not promote colony formation by NRK-49F cells or human fibroblasts in soft agar. In contrast, Transforming Growth Factor obtained from mouse embryo extract, did induce colonies of NRK fibroblasts but not human fibroblasts. IL-1 induced only a transient nonsignificant (p<0.1) down regulation of the EGF receptor on human fibroblasts after 4–6 hours of incubation as measured by 125I-EGF binding suggesting that IL-1 does not activate fibroblast proliferation through the EGF receptor, although a shared ligand may be present. IL-1 does not stimulate nonadherent colony formation of NRK or human fibroblasts in soft agar. IL-1 is a potent promoter of fibroblast growth in monolayer.

225. The Effect of Retinoic Acid on Normal and Transformed Human Embryonic Lung Fibroblasts. B. M. STANULIS-PRAEGER,* Department of Biology, Bryn Mawr College, Bryn Mawr, PA 19010

Retinoic acid at 10⁻⁹ M reduces growth rate and lowers saturation density of WI-38 and IMR-90 cells at all population doubling levels tested, and similarly inhibits the growth of SV-40 transformed WI-38 cells (VA13A). Hydrocortisone, which stimulates the growth of WI-38 and IMR-90 cells (VA13A). Hydrocortisone, which stimulates the growth of WI-38 and IMR-90 cells (VA13A). Hydrocortisone, which stimulates the growth of WI-38 and IMR-90 cells and inhibits growth of VA13A cells, significantly increases inhibition of VA13A cell growth when combined with retinoic acid. In WI-38 and IMR-90 cell populations, hydrocortisone combined with retinoic acid produces levels of growth intermediate between the levels achieved by untreated and retinoic acid treated cell cultures. The effects of retinoic acid do not show serum dependency. Viability of treated cells is 95–99% of controls. Seeding efficiency is not altered. Population life span (cumulative population doublings) of WI-38 cells is reduced by continuous exposure to retinoic acid, and old WI-38 cell populations are more sensitive to the effects of retinoic acid than young. VA13A cells treated with retinoic acid and retinoic acid combined with hydrocortisone show a reversion from the rounded, "cobblestone" appearance characteristic of the transformed phenotype to a more typical fibroblast-like conformation.

226. Response of Fibroblasts to Bleomycin Suggests Subpopulations with Variable Sensitivity.
228. F Prostaglandins Very Powerfully Stimulate Neonatal Hepatocyte Growth. U. ARMATO*,

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Some arachidonic acid derivative(s) (AAD) may be involved in the control of liver cell growth. We previously reported that an unidentified AAD (UAAD) set into active cycling ~75% of the hepatocytes in 4-day-old primary cultures of neonatal rat liver within 24 hours since the addition of AA (only 25% of the hepatocytes did cycle in the control cultures in the same lag) (1). PGE1, PGE, and PGE2, were stimulatory (when added separately) but did not reproduce the full extent of the UAAD effect (1). We now report that at low concentrations (e.g., 10^-9 mole/l), PGFl, and PGFla very intensely stimulated both the DNA-synthetic and mitotic activities of hepatocytes. On a 24-hour incubation, the action of PGFla by itself or in equimolar mixtures with other PG’s (e.g., A1, E1, E2) fully mimicked that of the UAAD. On a molar basis, PGFla and PGFla enhanced hepatocytes’ flow into S phase more powerfully than any other AAD so far tested, setting into active cycling ~95% of all the cultured hepatocytes; when used in equimolar mixtures with other PG’s, PGFla was at least as stimulatory as PGFla. These observations establish PGFla as quite powerful commitment factors for neonatal rat hepatocytes in culture. However, the understanding of the role of PGFla in the physiological control of hepatocytic proliferative activation must await the clarification of their interaction(s) with other AAD’s and various polypeptide growth factors (e.g., EGF, PDGF) possibly also involved in the process.

(1) Andreis, P. G.; Whitfield, J. F.; and Armato, U. Exp. Cell Res. 134: 265 (1981).
with a dermatome and examined the growth rates of fibroblasts derived from these discrete dermal sites. In Eagle's MEM supplemented with 10% fetal bovine serum, papillary fibroblasts show population doubling times (PDT) of 3.00 ± 0.52 days vs. PDT of 6.10 ± 2.0 days for reticular fibroblasts. These differences persist for at least 8 passages and 48 days in culture. We are currently determining whether differences in growth rate persist as the cells "age" in vitro. Our data confirm published reports from two laboratories (1,2) and are compatible with the presence of two fibroblast populations in the dermis with different growth potentials. The demonstrated heterogeneity in growth rates of papillary and reticular fibroblasts must be considered when skin biopsies are obtained to establish fibroblast cultures for the study of human disease since differences reported in growth rates, cell survival and cellular biochemistry may represent artifacts related to the type of fibroblast examined.

1) Harper, R. A., Grove, G., Science 204: 526, 1979.
2) Azzarone, B. and Macieira-Coelho, A., J. Cell Sci. 57: 117, 1982.

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231. A Liquid Microcarrier System. C. R. KEESE* and I. Giaever, General Electric Research & Development Center, P.O. Box 8, Schenectady, NY 12301

In 1964 Rosenberg demonstrated that both normal and transformed fibroblasts can be grown using as a substrate the phase boundary between culture medium and fluorocarbon fluids. We have found that the ability of anchorage-dependent cells to spread and divide on these substrates depends on the presence of an adsorbed protein layer with sufficient tensile strength. We have shown that this is dependent upon the presence of trace amounts of surface active (amphipathic) compounds, present in some fluorocarbon fluids, that presumably bridge between the fluorocarbon and the aqueous phase. Our understanding of these substances has been applied to devise a new type of microcarrier system in which cells are grown on the surface of small droplets (200 μ diam.) of emulsified fluorocarbons. Due to the increased surface area of this configuration, it is necessary to supplement the fluorocarbon fluid with a surface active additive to obtain good cell growth. To date, best success has been obtained using pentafluorobenzyl chloride as the additive. The liquid microcarrier system has been used to obtain logarithmic growth of both murine (3T3-L1) and human (MRC-5) fibroblasts. In both this microcarrier system and with growth of cells on planar phase boundaries the cell mass may be mechanically harvested without the use of proteolytic enzymes or chelating agents obviating the uncertain effects of such treatments.

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232. Laminin and Fibronectin Adsorption to a New Surface for Cell Culture: Effect on Cell Attachment and Spreading. T. A. GUHL* and D. M. Umstatter*, Becton-Dickinson Labware, Biological Research, Oxnard, CA 93030

A new polystyrene surface for cell culture was evaluated for its ability to support cell attachment and spreading after pretreatment with laminin and fibronectin. This new cell stratum called Primaria, recently introduced by Falcon Labware, is highly nitrogenated, possessing a surface chemistry very similar to collagen. Cellular response to this surface has been markedly different than to standard tissue culture (TC) plastic, so the binding of these cellular attachment factors to Primaria was investigated to determine possible mechanisms of this altered cellular interaction with the new surface. Baby hamster kidney (BHK) and PAM-212 mouse epidermal cells were evaluated for their ability to attach and spread of Primaria pretreated with either fibronectin, laminin, collagen IV, or a combination of these. 20% to 50% more fibronectin was bound to Primaria versus TC plastic, and complete BHK cell spreading occurred at one-half the input concentration. BHK cells were not responsive to collagen IV, but when the surfaces were coated with a collagen IV-laminin complex, nearly complete cell spreading on Primaria and very little spreading on TC plastic was observed. Laminin adsorption was equal on both surfaces, but PAM-212 cell spreading was more complete on laminin-coated Primaria. This study indicates a conformational effect, possibly the collagen-like chemistry of Primaria is binding laminin in a manner to expose more active cell-binding regions of the molecule.

233. Propagating Rat Liver Epithelial Cells on Microcarriers either in Serum-Supplemented or in Serum-Free Medium. M. CHESSEBEUF*, G. Mignot and P. Padiou*, INSERM U 208, Faculté de Médecine, 7 bd Jeanne d’Arc, 21033 DIJON Cedex — France

Growth characteristics of rat liver epithelial cells anchored on microcarriers were investigated either in serum supplemented medium (SSM) or in serum-free medium (SFM) prior to using microcarriers for production scale culture. Newly initiated and transformed SSM or SFM cell lines adapted to plastic surfaces were cultivated on Cytodex I® microcarriers for 10-15 days before subcultivation. Type, age and inoculum size of the cell lines were the most critical growth parameters. Specifically we observed a 3-5X increase in cell number with SFM cell lines and a 5-20X increase with SSM cell lines. Hepatic functions such as t-tyrosine aminotransferase induction by glucocorticoids or albumin and transferrin biosynthesis were maintained in such cultures. These data indicate that rat liver epithelial cell lines expressing hepatic functions are compatible with microcarrier cultures either in serum-supplemented or serum-free media and provide an efficient way for high-yield growth of differentiated hepatic cell lines.

234. Cinemicrographic Analysis of Mitosis in Primary Cultures of Adult Rat Hepatocytes. K. HASEGAWA, K. Watanabe and M. Koga, Dept. Physiol. Dokkyo Univ. Sch. Med. Mibu, Tochigi 321-02, JAPAN. (sponsored by Y. Yasumura*)

We have established the conditions for induction of mitosis in primary cultures of adult rat hepatocytes in a serum-free KL medium (B.B.R.C., 104, 259, 1982). When fibronectin (1-2 μg/ml), insulin (50 ng/ml) and
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235. Influence of Vessel Closures on Somatic Embryogenesis in Wild Carrot, Daucus carota L.
D. C. Verma* and J. D. Litvay, The Institute of Paper Chemistry, Appleton, WI 54912

The use of cotton plugs as closures for plant cell culture systems has served well for three reasons: (1) cotton plugs are chemically inert and safe; (2) they allow adequate gaseous exchange of the culture with the outside; and (3) they keep the cultures free from contamination. However, they are tedious to make. As alternatives, one can use a variety of closures available in today’s market. We examined their suitability for supporting somatic embryogenesis in wild carrot when we found that the closures we were using, i.e., polyurethane foam plugs on Erlenmeyer flasks, were toxic. Other closures — namely, silicone plugs, aluminum foil, and Morton’s stainless steel — proved satisfactory. Screw caps were satisfactory only when kept loose; if kept tight, CO₂ built up in the culture vessels and impaired embryogenesis development. The above-reported toxicity of the polyurethane plus was admitted to be a possibility by its manufacturer who said, however, that it happened only rarely when the plugs were in place during autoclaving of the culture medium.

236. Requirement for Endogenous Collagen Synthesis in Adhesion of Epithelial Cells to Collagen Substratum.
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We previously reported that fibroblast conditioned serum-free medium contained growth regulating proteins (PCM) for rat liver epithelial cells (RL-34) on the substratum with type I collagen (Vitrogen 100). The adhesion promoting factors were proteins with molecular weights of 30K to 70K but not fibronectin. We also found that when cycloheximide was added to PCM medium, adhesion of RL-34 to collagen substratum was inhibited, indicating that de novo synthesis of proteins was required for adhesion. We studied the role of endogenous collagen synthesis in adhesion of RL-34 to type I collagen substratum with 100 μg/ml PCM or 25 μg/ml partially purified adhesion-promoting factor using cis-hydroxyproline (CHP), a collagen synthesis inhibitor. We obtained the following results: 1) CHP inhibited adhesion of RL-34 to type I collagen substratum in a dose dependent manner at >100 μg/ml although 25 μg/ml CHP had no effect, 2) neither proline nor trans-4-hydroxyproline influenced adhesion, suggesting that the effect of CHP was not due to an inhibitory effect of imino acids, and 3) the addition of proline at a concentration equal to that of CHP restored adhesion. These data demonstrate the requirement for endogenous collagen synthesis in adhesion of RL-34 to type I collagen substratum with PCM, and suggest that the endogenous collagen may be different from type I collagen used as substratum.