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ABSTRACTS FROM TWENTY-FIRST ANNUAL MEETING

1. **Special Symposium,** *organized and chaired by* R. T. Christian (University of Cincinnati) and D. Pace (University of the Pacific).
Environmental Biology and Tissue Culture.

2. **The Action of Pesticides on Primary Cell Cultures and Established Cell Lines.** B. W. Wilson (University of California, Davis, California).

3. **Differential Sensitivity of Chemical Carcinogens in vitro.** J. A. Di Paolo (National Institutes of Health, Bethesda Maryland).

4. **Influence of Environmental Agents on Pulmonary Macrophages.** D. Coffin and D. A. Gardner (National Air Pollution Control Administration, Cincinnati, Ohio).

5. **The Use of Tissue Culture to Detect Viruses in Water.** P. Ludovici (University of Arizona, Tucson, Arizona).

6. **Special Session,** *Organized and chaired by* W. B. Savchuk (National Institutes of Health, Bethesda, Maryland).
Research Support for Cell and Tissue Culture.

7. **Panel on Research Support for Cell and Tissue Culture.** G. C. Wood (American Cancer Society, New York, New York).

8. **Panel on Research Support for Cell and Tissue Culture.** V. Apgar (National Foundation-March of Dimes, New York, New York).

9. **Panel on Research Support for Cell and Tissue Culture.** J. F. Herndon (National Cystic Fibrosis Research Foundation, New York, New York).

10. **Panel on Research Support for Cell and Tissue Culture.** E. E. Clark (National Science Foundation, Washington, D. C.).

11. **Panel on Research Support for Cell and Tissue Culture.** J. S. Kirby-Smith (Atomic Energy Commission, Washington, D. C.).

12. **Panel on Research Support for Cell and Tissue Culture.** D. F. Flick (Food and Drug Administration, Washington, D. C.).

13. **Panel on Research Support for Cell and Tissue Culture.** K. R. Dirks (U. S. Army Medical Research and Development Command, Washington, D. C.).

14. **Panel on Research Support for Cell and Tissue Culture.** D. T. Chalkley (National Institutes of Health, Bethesda, Maryland).

15. **Panel on Research Support for Cell and Tissue Culture.** D. G. Murphy (National Institutes of Health, Bethesda, Maryland).

16. **Panel on Research Support for Cell and Tissue Culture.** A. E. Heming (National Institutes of Health, Bethesda, Maryland).

17. **Panel on Research Support for Cell and Tissue Culture.** P. G. Stansly, (National Institutes of Health, Bethesda, Maryland).

18. **Panel on Research Support for Cell and Tissue Culture.** W. G. Moss (National Institutes of Health, Bethesda, Maryland).

19. **Session in Depth: Hormones as Signals and Hormones as Products of Animal Cells in Culture.** *chairman:* A. T. Tashjian (Harvard school of Dental Medicine, Boston, Massachusetts).

20. **Control by Steroid Hormones Mammalian Gene Expression.** D. W. Martin, Jr. and G. M. Tomkins (University of California Medical Center, San Francisco, California 94122).

We are studying the mechanism of glucocorticoid-mediated enzyme induction using HTC cells, a line of rat hepatoma cells derived from Morris hepatoma 7288c. In these cells, glucocorticoids induce a 5- to 20- or 30-fold increase in the rate of synthesis of the enzyme tyrosine aminotransferase (TAT) without affecting growth rate or general macromolecular synthesis or degradation. Studies using populations of random and synchronized cells have led to a general model for gene regulation which entails two genes (structural and regulatory) and two configurations of their activity, active and inactive. The model proposes that a cell cycle-dependent, but inducer-independent, event controls the transcription of the structural and regulatory genes. The inducer functions by modulating enzyme synthesis mechanism involving a labile posttranscriptional repressor of messenger RNA function. Experimental verification of the model and certain predictions made by it will be discussed.

21. **Control of the Production of Two Protein Hormones by a Clonal Strain of Rat Pituitary Cells.** F. C. Bancroft and A. H. Tashjian, Jr. (Harvard School of Dental Medicine and Harvard Medical School, Boston, Massachusetts).

The GH_3 strain of cells produces (i.e., synthesizes and secretes) both growth hormone (GH) and prolactin (P). Studies of the effects of external

stimuli have indicated that, in spite of the physical similarity of these protein hormones (each is a single polypeptide of molecular weight ~20,000), their production is controlled by different mechanisms. Addition of hydrocortisone (HC) (3×10^{-6} M) to the growth medium leads, after a lag of 12 to 24 hr, to an increased relative rate (rate in experimental cells divided by rate in control cells) of GH production. The relative rate reaches a maximum of 5 to 8 at 70 to 100 hr. Stimulation by HC of GH production is observed in cells growing in a number of different media and in either the stationary or the exponential phase of growth. Indirect estimates indicate that in exponentially growing cells, GH represents about 2% and 14% of the total protein synthesized by control and fully stimulated cells, respectively. Maintenance of the stimulated state requires HC. HC decreases both the growth rate of GH_3 cells, and their incorporation of amino acids into acid-insoluble material. At the same time that HC stimulates GH production, it decreases the relative rate of P production to about 0.2 to 0.3. On the other hand, addition of acid extracts of bovine hypothalamus, cerebral cortex, kidney, or liver (0.3 to 1.0 mg protein per ml) to the medium leads to an increase of the relative rate of P production to 6–9, while decreasing the relative rate of GH production to about 0.5. GH_3 cells have recently been adapted to grow in suspension culture, and effects of HC are similar to those observed in monolayer.

22. Development of Hormone-dependent Cell Strains. M. Posner (University of Massachusetts, Boston) and W. Gartland and G. Sato (University of California, San Diego, California). Most types of animal cells in situ require trophic hormones for maintenance and growth. Up to the present time no hormone dependence has been reported for established tissue culture strains. The object of our experiments is to develop such strains. To this end, cells from normal tissue and from hormone-dependent tumors have been put into culture in the presence of their respective trophic hormones. From time to time, the cells are transferred to a medium deficient in the appropriate hormone. This has usually been accomplished by using as a serum component of the medium, serum from hypophysectomized animals. Under these conditions only autonomous cells are able to multiply and are selectly killed by the addition of BUDR. Survivors of this treatment should be hormone-dependent. In this way mammary cells have been developed which will not grow in medium composed of hypopysectomized dog serum and F10 medium. These cells will grow, however, if the medium is supplemented with hypophysectomized dog medium taken from a strain of rat pituitary cells which secrete growth hormone and prolactin. These cells will also grow if the hormone-deficient medium is supplemented with growth hormone, prolactin, and insulin. Progress on the development of other hormone-dependent cell lines will be reported.

23. Nuclear-Cytoplasmic Exchange of RNA and Protein—The Role of Serum. Harold Amos, Michel Horisberger, and Richard Hoyt (Harvard Medical School, Boston, Massachusetts). Radioautography and cell fractionation studies have produced evidence that at 27°C RNA synthesized in the nucleus and nucleolus migrates more slowly into the cytoplasm than at 37°C. If at 37°C serum is removed from primary chick cells, labeled RNA accumulates neither in nucleoli nor in the cytoplasm of the cells (Horisberger and Amos, in press). Evidence has been obtained indicating that raising the temperature from 27°C to 37°C or the addition or removal of serum results in immediately detectable effects on RNA distribution. The serum fraction, hopefully a single class of protein, responsible for inducing RNA transport to the cytoplasm will be identified. RNA synthesis and processing are altered by the addition of serum macromolecules. HeLa cells do not respond in the same way to serum removal.

24. The Effects of ACTH on the Metabolism of Adrenal Cell Cultures. J. Kowal (The Mount Sinai School of Medicine, New York, New York). Monolayer cultures of ACTH-responsive mouse adrenal cortex tumor cells provide a means for the study in vitro of the mechanisms of the immediate steroidogenic response to ACTH and the metabolic consequences of long term stimulation. As much as 10-fold increases in steroid output can be elicited by ACTH, cyclic AMP, and cyclic CMP. Cyclic GMP, IMP, and UMP are inert. In certain lines other adenosine nucleotides are also steroidogenic. ACTH increases the incorporation of acetate and glucose into steroids. This involves the rapid turnover of a small pool of cholesterol which is under feedback control. Glycolysis is increased by ACTH. ACTH-induced alterations in the intracellular levels of the glycolytic intermediates suggest an activation of phosphofructokinase and hexokinase. However, this increase in glycolytic activity does not trigger the steroidogenic response, since it can occur in the absence of glucose or when glycolysis is inhibited with 2-deoxyglucose. ACTH does not increase amino acid incorporation into proteins, or uridine into RNA. However, if ^{32}P is added to the medium, ACTH and cyclic AMP increase the incorporation of ^{32}P into protein, phospholipids, and RNA. There are comparable increases in labeling of ATP but the total ATP content is unaffected. This effect of ACTH and its stimulation of glycolysis are not inhibited by cycloheximide or puromycin, compounds which profoundly inhibit steroidogenesis in resting and stimulated cells. The data suggest that the steroidogenic action of ACTH is one of many alterations in adrenal cell metabolism induced by this peptide.

25. The Pineal Gland in Organ Culture: Regulation of Melatonin Production D. C. Klein, G. R. Berg, and J. Weller (Laboratory of Biomedical Sciences, National Institute of Child Health and Human Development, Bethesda, Maryland).

Pineal glands obtained from immature and adult rats continue to produce melatonin (N-acetyl 5-methoxytryptamine), for up to 6 days in organ culture. Glands are incubated at the interface of a chemically defined media and a 95% oxygen-5% CO_2 gassing atmosphere. The addition of norepinephrine (10^{-4} to 10^{-6} M) to culture media stimulates the conversion of radiolabeled tryptophan or 5-hydroxytryptamine to melatonin by pineal glands. This effect is apparently mediated by adenyl cyclase. Like norepinephrine, dibutyryl cyclic AMP and theophylline stimulate melatonin production 7- to 10-fold. Following a 1.5- to 18-hr treatment of cultured pineals with norepinephrine or dibutyryl cyclic AMP, N-acetyltransferase activity is also stimulated. This is first of two enzymes in the conversion of 5-hydroxytryptamine to melatonin. Treatment with other catecholamines, but not histamine, tryptamine, 5-hydroxytryptamine, putrescine, ethanolamine or tyramine, results in 7- to 20-fold stimulation of the activity of N-acetyltransferase as measured in broken cell homogenates. The addition of cycloheximide with either norepinephrine or dibutyryl cyclic AMP prevents the stimulation of enzyme activity and melatonin production. No effect of these compounds on N-acetyltransferase activity is observed when they are added directly to enzyme assays. These studies indicate that melatonin synthesis can be regulated by norepinephrine through a cyclic AMP mechanism which regulates the activity of N-acetyltransferase and requires continual protein synthesis. Melatonin production is secondarily increased by mass action resulting from the increased production of N-acetyl 5-hydroxytryptamine.

26. Control Mechanism for Gonadotropin Production in Vitro. R. A. Pattillo, G. O. Gey, E. Delfs, W. Y. Huang, R. O. Hussa, R. F. Mattingly, and A. C. F. Ruckert (Marquette School of Medicine, Milwaukee, Wisconsin and Johns Hopkins Medical School, Baltimore, Maryland).
The human trophoblast cell line, BeWo (Pattillo and Gey), derived from gestational choriocarcinoma and initially transplanted to the hamster cheek pouch by Hertz, has been shown to produce a variety of placental hormones in uniform, stable, and predictable quantities. Production of HCG (human chorionic gonadotropin), HPL (human placental lactogen), progesterone, estrone, and estradiol is present in high yield. Precursor studies show progestational steroid production to depend upon the presence of pregnenolone in the incubation medium; estrone and estradiol require dehydroepiandrosterone and Δ^4-androstenedione. HCG production yields 1000 IU per 10^8 cells per 24 hr. Using this model, immature rat uterine weight bioassays reveal inhibition of HCG production when the cells are incubated with either progesterone or pregnenolone. This is the first report of gonadotropin hormone control by feedback inhibition from endogenously synthesized steroids. The trophoblasts cell system has proved readily adaptable for study of multiple synthetic functions and controls in vitro.

27. Contributed Paper Session: General Topics and Methodology. *Chairmen:* W. Stinebring and J. A. Sykes.

28. Short Term Preservation of Bone Marrow at −20°C for Culture in Wells. E. H. Reisner, Jr. (St. Luke's Hospital Center, New York, New York 10025).
Previous attempts to culture marrow preserved by freezing using a variety of techniques had been unsuccessful. In a mixture of 30 g of methanol and 10 g of PVP (Kliman, A. Transfusion, *4:* 281, 1964), explants of marrow obtained from ribs of patients undergoing lung surgery were preserved in a standard icebox freezer (−20°C) in Petri dishes for from 2 to 7 days. They were then cultured in wells (13 mm × 10 mm) for 6 to 7 days in the presence of erythrostimulatory or granulostimulatory factors, in a medium of 20% human serum and 80% NCTC 109. Erythropoiesis and granulopoiesis were measured by explant uptake of ^{59}Fe and ^{35}S sodium sulfate, respectively. Controls were grown with pooled human serum. The performance of the preserved marrow was compared with that of the same marrow tested under comparable conditions when fresh. While in some cases the uptake of the preserved marrow was less than when it was fresh the ratio of increase in radioactivity of stimulated cultures to controls was similar. These experiments indicate that lowering the metabolic activity of cells by reducing the temperature in a medium that prevents freezing will allow the survival of a sufficient number of stem cells to permit the use of thus stored marrow for in vitro study, at the investigator's convenience a few days later. Since the preparation of cultures with fresh marrow is dependent on the vagaries of the operating room schedule, the ability to preserve marrow for later use is a tremendous help in permitting the regular, orderly planning of experimental designs.

29. Preliminary Studies of a New Epithelioid Cell Line of Human Origin. J. A. Sykes, J. Whitescarver, J. T. Nolan, and P. Jernstrom (Southern California Cancer Center, California Hospital Medical Center, Los Angeles, California 90015).
A new human cell line (ME-180) has been established from an omental metastasis of a cervical carcinoma. The patient suffered from a primary carcinoma of the cervix uteri which metastasized very rapidly despite surgery, X-ray therapy, and application of radium implants. The cells are epithelial in character showing frequent intercellular bridges and in older cultures desmosomes and cytoplasmic tonofibrillae. Chromosome numbers range from 48 to 130 with a subtriploid mode. Simple additions to various groups were seen, as were chromosomal fragmentation, dicentrics, and other rearrangements characteristic of transformed cells.

In a few instances marker chromosomes were present but formed no stem line. ME-180 is a heteroploid cell line with an unstable karyotype and a subtriploid mode. Growth curve studies at passages 45 and 75 gave almost identical results. The population doubling time using Eagle's minimum essential medium (MEM) containing 30% fetal calf serum was 16 to 24 hr. The mitotic index was 9.86 (determined at 24 and 48 hr). The absolute plating efficiency using MEM containing 30% fetal calf serum was 48.69. ME-180 cells readily form carrier cultures with influenza and other myxoviruses. The viral sensitivity of ME-180 to other viruses was different to the HeLa cells studied in parallel. Several clones have been derived from the parent culture and are being investigated.

30. Isolation and Cultivation of Rat Fetal Liver Cells. S. S. El Kadi and A. N. Stroud (Louisiana State University Medical Center, New Orleans, Louisiana, and Pasadena Foundation for Medical Research, Pasadena, California).

There have been reports published about the preparation of liver cells for long term maintenance and subcultivation. In many cases, questions have arisen with regard to the morphological and functional health of such cells. On the basis of numerous experiments monitored by phase contrast and cinematographic observations, we recommend the following techniques for isolation, maintenance in vitro, and subcultivation of liver parenchymal cell populations. Finely minced liver fragments from 16-day rat embryos were dispersed in 0.3% collagenase in Hanks' BSS plus 20% fetal calf serum for four successive periods of 10 min each, followed by thorough washing and suspension for a final hour at 4°C in growth medium to give a final concentration of approximately 2×10^6 cells. The growth medium consists of Eagle's MEM, fetal calf serum (10%), vitamins (2×), nonessential and essential amino acids (2×), and hydrocortisone (0.02%, suggested by C. Waymouth). The first subculturing of the monolayer was made at 10 days, and then at 2-week intervals thereafter. Cellular dispersal was made in a mixture of collagenase (0.05%) and hyaluronidase (0.10%) for 15 to 30 min. Cells grown in this way give a doubling time of 4 days. Using the above methods, we have been able to keep cells growing for more than 2 months.

31. Methods to Reduce Contamination of Cell Cultures. Gerard J. McGarrity and Lewis L. Coriell (Institute for Medical Research, Copewood Street, Camden, New Jersey).

The most frequent causes of contamination are techniques, housekeeping procedures, unsterile supplies, and culture media. The most common contaminants are *Mycoplasma, Torulopsis* sp., diphtheroids, and *Pseudomonas*. Routine procedures adopted in this laboratory have almost eliminated accidental contamination and they will be described and documented, including: uses of HEPA-filtered air, media preparation and sterilization, sterility tests of serum, treatment of floors and flat surfaces, clothing, and asceptic procedures.

32. Cytological Effects Following Irradiation with a Far Ultraviolet Laser Wavelength.[1] T. Okigaki, D. E. Rounds, and K. S. Narayan (Pasadena Foundation for Medical Research, Pasadena, California).

The Don-C line of Chinese hamster cells was irradiated with laser power at 2650 Å. This wavelength was derived from the quadrupled frequency of a Q-switched neodymium laser. Cells were exposed to 20-nsec flashes either as suspensions or as monolayers on quartz coverslips. Although the photon distribution was inhomogeneous, the power density was estimated to be 0.1 mw per cm^2 per flash. Morphological changes included cytoplasmic vacuolization, peripheral extension and withdrawal of pseudopods, zeiosis, and nuclear pycnosis or irradiated cells. The cell population showed the greatest decrease at 48 hr postirradiation, but surviving cells showed a growth rate parallel to that of control cells. The mitotic index followed a similar pattern. The frequency of multinucleate cells, and cells with multilobular nuclei or micronuclei showed a 5-fold increase at 48 hr postirradiation, and persisted at a high level even on the 8th day. Although a variety of chromosomal anomalies were produced, cells which survived 5 days showed primarily dicentrics and endoreduplications.

33. Observation of Mammalian Cells in Vitro and Cell Culture Contaminants by Special Microscopic Techniques. J. Fogh (Sloan-Kettering Institute, New York, New York).

Morphological studies of cells in vitro have, in the past, mainly depended on observations by bright field microscopy of fixed and stained cultures or phase microscopy of living, unfixed cultures. It has become apparent that special microscopic techniques of either unfixed or fixed preparations may bring out details of the cells or of intercellular relationships which are not well, or not all, observed by the other techniques. Routine fixation techniques seem to be particularly damaging to the peripheral cytoplasm and intercellular connections between the glass-attached cells. The advantages in supplementing morphological studies with the application of special microscopic techniques will be demonstrated with micrographs of cultured human cells under various culture conditions and in mixed culture. They have been observed with interference microscopy and interference-contrast (Nomarski) microscopy at the single cell level or in mass culture. In addition, examples of various types of cell culture contamination, their effects on cell morphology, and details of the contaminants

[1] This work was supported by the U. S. Army Medical Research and Development Command, Office of the Surgeon General, under Contract DA-49-193-MD-2564.

have been examined by these techniques and will be presented.

34. Observations of Infant Brain Cells Cultured in Vitro. D. AMSTERDAM, S. E. BROOKS, M. ADACHI, AND B. W. VOLK (Isaac Albert Research Institute, Kingsbrook Jewish Medical Center, Brooklyn, New York).

Observations were made over a 21-day period of the outgrowth of explanted brain tissue obtained by frontal lobe biopsy from a 10-week old infant with proven Tay-Sachs disease (a neuronal lipid storage abnormality). The tissue obtained was collected in Hanks' balanced salt solution, rinsed, and then minced into 2-mm fragments. These explants were impressed directly into plastic T-30 flasks without the benefit of plasma or collagen clot. Flasks were overlayed with minimal (2 ml) Eagle's MEM containing nonessential amino acids and 10% fetal calf serum; antibiotics, penicillin, and streptomycin were added to half of the flasks. Experience had shown that the above medium was superior to MEM containing 20% human serum, or a medium consisting of equal aliquots of beef amniotic fluid, bovine serum ultrafiltrate, human serum, and Hanks' balanced salt solution. Cells appeared in about 3 days and developed equally well in medium containing antibiotics as compared to the drug-deficient medium. As determined from phase contrast observations, macrophages appeared prior to another cell type which morphologically resembled glial cells, probably astrocytes. The latter displayed many cytoplasmic extensions giving rise to a stellate structure. Unlike the only other study reported, we found some evidence of glial elements. The ability to maintain Tay-Sachs brain cells *in vitro* provides a means for the study of the ultrastructural and biochemical changes which characterize this disease state.

35. Cultivation of Isolated Mammalian Ovarian Follicles. H. S. GROB (New York University, New York, New York).

Isolated follicles obtained by enzymatic dispersal of ovaries from mice, rats, rabbits, and cats have been cultivated in a variety of culture systems. Follicles obtained from the dispersal are structurally intact and capable of growth and hormone production when cultivated in vivo. These intact follicles exhibit histotypical growth when cultured in vitro with a variety of commonly available culture media. Upon attachment to the growing surface, the follicle cells grow out and the oocyte is released to the medium where it ultimately undergoes degenerative fragmentation. Outgrowths obtained from the follicles are epithelioid and typically contain two cell types which differ in size and staining characteristics. Addition of gonadotrophins to the medium results in increased mitotic activity. These data are consistent with previously reported data from in vivo and organ culture studies. The monolayer cultures can be stimulated to produce steroid hormones for limited periods after initiation of the cultures. Organotypic growth of the isolated follicles has been achieved utilizing modified culture procedures and specific hormone additions to the medium. In these systems the follicles grow, mature, and produce steroids in a manner comparable with in situ development. Such systems may be useful in attempting to elucidate the mechanisms which govern ovarian follicle maturation.

36. Rolling Bottle Perfusion Cultures. P. F. KRUSE, JR. AND W. WHITTLE (The Noble Foundation, Inc., Ardmore, Oklahoma 73401).

Since living systems are open systems, it is a curious fact that studies of them in vitro have almost exclusively been done with closed culture techniques. This report describes a rolling bottle perfusion culture apparatus, in which open system conditions of steady state kinetics are approached. Bottle cultures were fitted with Swivel Caps (New Brunswick Scientific Company) containing influent and effluent medium conduits. Medium was fed by gravity and controlled with a compressed air clamp and recycling timers to vary both the time intervals between feedings and the quantity of each feeding. With 290 mm $\times$ 100 mm diameter bottles, 730 cm^2 inside surface, and 12 rph rotation speed, these data were obtained (cell type, time in days, initial and final cell counts per bottle, and estimates of number of cell layers with confluency = 1.0): WI-38VA13A, 13.8, 30.7 $\times 10^6$ to 1.52 $\times 10^9$, 0.25 to 12.5. NF-1 diploid human fibroblasts, 17.1, 8.94 $\times 10^6$ to 187.6 $\times 10^6$, 0.16 to 3.6. Jensen rat sarcoma, 9.9, 5.66 $\times 10^6$ to 1.62 $\times 10^9$, 0.01 to 7.0. Final Jensen cell count represents approximately 4 g of cell pack per bottle. At bottle rotation speeds of 12, 26, 52, and 160 rph total yields of Jensen cells were equivalent, but at 12 and 160 rph speeds, floating cells were 6 and 31% of the total, respectively. At 26 and 52 rph > 99% of the cells were sticking in multiple layers. Similar tests determined the effect of varying perfusion rates. High cell yields and steady state environments are among the attributes of this culture system.

37. Methods of Studying Cell Aggregates in Culture—Morphology, EM Studies, Chromosomes. A. FJELDE, R. ZEIGEL, AND J. GRACE (Roswell Park Memorial Institute, Buffalo, New York).

Cells proliferating in clusters are sometimes difficult to characterize. The authors wish to describe methods and new results obtained for three different types of cells and culture as follows: established human hematopoietic lines in stationary bottles, a mosquito line in a spinner system, and hematopoietic cells in agar. EM and light microscope studies indicated that, in cultures of human thoracic duct cells, a transformation of simple lymphocytes occurred. These were compared with cultures derived from the total cell population found in peripheral blood. Fractions containing different cell types could be collected. Mosquito

cells with characteristic chromosomes grew well in a spinner system in which aggregates at the fluid-air interface released cells into the medium. A modification of the acid-orcein squash technique was useful in characterizing clusters of cells grown in agar. The morphological studies could be done in conjunction with immunological studies. A suitable cell cluster was removed from the agar with a fine pipette with just enough BSS to ensure proper spreading under a cover slip with or without dye. After squashing, the stained cells were ready for study. The unstained preparations were frozen on Dry Ice, and the coverslip and slide were separated. After drying, the preparations were stained or treated for special work. The squash method afforded a convenient way of combining studies of chromosomes, morphology, immunological activity, mixed cultures, and attempts to produce hybrids. Although extensive interdigitation occurred in cell aggregates, rare instances of shared common membranes were seen in EM or light microscope studies.

38. Combined Sera Supplementation and Optimal Proliferation of Diploid Fibroblast-like Cells Differing in Experimental Origin. G. YERGANIAN (The Children's Cancer Research Foundation, Boston).

Normal, malignant, and virus-transformed fibroblast-like derivatives of the Chinese hamster retain the classic diploid chromosome number ($2n = 22$) for 85 to 125 passages. Yet, irregularities in the growth rates of the two former cell types hinder molecular approaches to establish comparative biochemical baselines prior to the onset of aneuploidization. By employing varying proportions of sera types (fetal calf, calf, dialyzed calf, agamma calf, and horse) in a modified Eagle's MEM, cell membrane features of nonviral associated cell types are altered and, in turn, are reflected by improved rates of proliferation. Permanent cell lines develop by the fourth passage or some 25 days in vitro, a feature which previously required 14 to 20 passages to achieve with single serum-supplemented media. Difficult to propagate diploid malignant derivatives, which exhibit contact inhibition, are now cultured routinely for the first time in 12 years. Biochemical studies on mass cultures are now feasible by the seventh passage after biopsy. The favorable effects of combined sera are even more dramatically reflected by cells derived from other hamster species (Armenian and Transcaucasian). Previously, cells from the former species failed to survive beyond the fourth passage unless transformed by a virus and experiencing immediate aneuploidization. Recent trials with combined sera have yielded diploid cell lines exhibiting maximum proliferation once the fourth and most critical passage is surpassed. The proportions of combined sera are altered when culturing cells in roller flasks.

39. Effects of Collagen and Acid Polysaccharides on the Growth of Cells in Semisolid Media. F. K. SANDERS (Sloan-Kettering Institute for Cancer Research, New York).

Growth in soft agar has been used to assay transformation by polyoma, SV40, and RSV, and is generally assumed to be a property of transformed rather than normal cells. The substitution of agarose, or carboxymethylcellulose (CMC), for agar, enhances the ability of untransformed cells to grow. Acid polysaccharides such as heparin, dextran sulfate, ovomucoid, or chondroitin sulfate can suppress the ability of untransformed cells to grow in semisolid media, while transformed cells remain unaffected. The addition of native collagen to gel suspension media increases the colony-forming ability of untransformed cells about 5-fold but not that of transformed cells. When collagen is used to coat an inert polycarbonate porous membrane lying between two gel layers, cells spread out on the collagen, and form colonies of distinctive morphology. Membranes bearing colonies that have developed under CMC gels can be recovered by simply chilling the plates; the colonies can be cut out of the membrane with scissors and the cells removed with a drop of collagenase for subsequent recloning.

40. Growth of L Cells in a Chemically Defined Medium in a Controlled Environment Culture System. G. W. TAYLOR, J. P. KONDIG, S. C. NAGLE, JR., AND K. HIGUCHI (Biological Sciences Laboratories, Department of the Army, Fort Detrick, Frederick, Maryland).

Equipment has been developed to permit monitoring and automated control of environmental variables such as pH, temperature, pO_2, pCO_2 and oxidation-reduction potential in order to study their effects on the growth and metabolism of cultured mammalian cells. A battery of six water-jacketed 500-ml Bellco spinner flasks was instrumented to provide (by electrode probes) information on pH, pO_2, and oxidation-reduction potential of each culture during growth. Devices such as stepping switches and motorized valves coupled to the sensing probes permitted control of environmental conditions. Studies with automated control of pO_2 levels in L cell cultures showed that dissolved O_2 tension of approximately 9% was optimal for cell growth. At pO_2 values of 5% and 20%, peak cell yields as well as growth rates were reduced by approximately 20%. Peak yields of L cell cultures exceeded 5×10^6 cells per ml when grown for 4 days without medium renewal from inocula of approximately 1.0×10^6 cells per ml in a defined medium sparged with 5% CO_2 and adequate O_2 to maintain 9% dissolved O_2 tension. The oxidation-reduction potentials of L cell cultures reflected the pO_2 levels in the medium and ranged from -25 to $+150$ mv (calomel reference) for O_2 values ranging from 2 to 20% dissolved oxygen tension.

41. Improved Medium for the Cultivation of Differentiated Liver Cells A. RICHTER (Laboratory Cell Suppliers, Frederick, Maryland 21701).

An improved medium for the cultivation of dif-

ferentiated rat cell cultures isolated by Coon (J. Cell Biol., *39:* 29a, 1968) was developed by adding to Eagle's minimum essential medium (MEM) 10 essential ingredients not previously present in that recipe and 1% fetal calf serum. If one uses with the new medium a trypsin concentration of 0.005% crystalline trypsin or 0.05% crude trypsin for splitting, the cultures grow at the same rate or better than in Eagles MEM + 10% fetal calf serum. This demonstrates that (a) presently used concentrations of trypsin and serum for standard tissue culture work are excessive, (b) both undefined components of the culture-harvest system can be reduced in concentration if an improved medium is employed, and (c) 10 of the ingredients supplied by serum may be described as asparagine, serine, pyruvate, iron, zinc, biotin, vitamin B_{12}, linoleic acid, lipoic acid, and putrescine. The remaining function of the serum is (a) to provide antitryptic activity and (b) to supply further nutritional ingredients still presently unknown. The improved medium can also be used for the growth of cells other than the rat liver cell cultures, including human diploid foreskin cells, L cells HeLa cells, WI-38, BHK 21 both before and after polyoma transformation, human lymphocytoid cells, and primary chick embryo cells. Exact formulation will be provided.

42. Contributed Paper Session. Morphology and Differentiation. *Chairmen:* C. J. DAWE AND M. HARDY.

43. The Effects of Parathyroid Hormone, Calcitonin, Dibutyryl Cyclic AMP, and Imidazole on Bone in Organ Culture. J. N. M. HEERSCHE, M. P. M. HERRMANN-ERLEE, AND P. J. GAILLARD (Institute of Cell Biology and Histology, University of Leiden, The Netherlands).

Parathyroid hormone (PTH) and calcitonin (CT) each produce characteristic histological and biochemical changes in cultivated bone rudiments. In the present investigation, we compared the effects of these hormones with those of dibutyryl cyclic adenosine monophosphate (DCAMP) and imidazole, two substances which mimic in vivo the response to PTH and CT. Histological effects were studied in explanted radii of 15-day-old mouse embryos. PTH and DCAMP produced similar changes in all three tissue compartments studied, bone, cartilage, and connective tissue inside the shaft. CT and imidazole both affected bone causing an increase in the number of active osteoblasts inside the shaft. Bone demineralization and the release of lysosomal β-glucuronidase were studied in explanted calvaria from full term rat and mouse embryos. PTH and DCAMP both increased bone demineralization and the release of β-glucuronidase. The response to DCAMP, however, was less pronounced and became apparent only after 2 days of cultivation. CT and imidazole both inhibited bone demineralization, but the release of β-glucuronidase appeared to be inhibited only by CT. These observations provide further evidence that the action of PTH on bone is mediated by cyclic AMP. Since the effects of CT and imidazole on the population of bone cells were similar, it seems possible that these agents inhibit bone mobilization through a common cellular mechanism.

44. Morphology of Developing Avian Central Nervous Tissue in Culture. S. U. KIM AND Y. TANAKA (Department of Anatomy, University of Saskatchewan, Saskatoon, Canada).

Little work has been reported on the avian CNS in tissue culture. Therefore, an attempt was made to establish standardized cultures of chick embryo CNS. Various parts of brain, i.e., cerebrum, cerebellum, optic tectum, spinal cord, and retina, from 8- to 12-day-old chick embryos were grown either in Maximow's double coverslip assemblies or in flying coverslip roller tubes. The onset of myelination occurred about the 7th day of culture and myelination continued until the end of the 3rd week. During the 2nd week the explants spread and neurons could be observed. In cultures impregnated with silver by Bodian's method, pyramidal cells of cerebrum, Purkinje cells of cerebellum, motor neurons of spinal cord, ganglion and bipolar cells of retina could be clearly seen. The electron micrographs of the cultures showed with remarkable similarity all the characteristic ultrastructures, including synapses, which can be seen in vivo.

45. The Effect of Glucagon on the Pancreatic Islet of Rat Primordium Grown in Vitro.[1] M. R. SCHWEISTHAL (University of Kentucky, Lexington, Kentucky).

The splenic portion of the pancreas from 17½-day embryos was explanted and grown on a liquid medium for 10 days. The medium consisted of equal parts of chick embryo extract and rooster serum. For experimental cultures the medium was injected with 3 μl of glucagon (Lilly) at explantation and at each 48-hr transfer. All cultures were incubated at 37°C in air supplemented for 1 hr daily with O_2 (95%) and CO_2 (5%). At the termination of the culture period the cultures were fixed in Bouin's solution, sectioned at 5 μ, and stained with aldehyde fuchsin, chrome alum hematoxylin-phloxin, or with chrome alum hematoxylin-aldehyde fuchsin (ponceau de xylidine or ponceau acid fuchsin counterstains). Beginning controls, prenatal and postnatal controls were treated similarly. Histological comparison showed both control and experimental cultures to have fewer peripheral islet cells than normal controls of comparable age. However, experimental cultures demonstrated a more pronounced reduction in peripheral islet cells; indeed, some of these cultures had islets which appeared to be composed entirely of β-cells. It is not yet clear whether this effect reflects the suppression of α-cell elaboration, destruction of the α-cell, or conversion of the α-cell to a β-cell. Our studies are being continued to include electron microscopic study of similarly treated tissue.

[1] Supported by National Institutes of Health Grant 2 R01 AM14119-20.

46. Acetylcholinesterase Isozymes in Primary Cultures of Chick Embryo Skeletal Muscle and Heart.[1] B. W. Wilson (University of California, Davis, California).

Chick embryo muscle contains three electrophoretic bands of acetylcholinesterase (AChE) activity. The slowest form corresponds to the single band of AChE activity found in chick embryo heart. The two fast forms disappear within 2 weeks after hatching. Their presence on acrylamide gels is associated with AChE activity in the muscle fiber sarcoplasm. Dispersed 11-day embryo pectoral muscle and heart cells were cultured in a medium containing Hanks' saline, medium 199, horse serum, and embryo extract. Myotube formation occurred within 3 days. The newly formed myotubes and myoblasts had little AChE activity. After 4 to 5 days the myotubes developed regions of AChE activity in their sarcoplasm. Gel electrophoresis showed that these cultures contained the AChE forms found in muscle in situ. Heart cells grown under the same conditions contained the AChE band characteristic of its tissue of origin. Growth but not myotube formation was inhibited by 5×10^{-5} M or 3×10^{-4} M Malathion or Malaoxon, and AChE activity of the myotubes was much decreased. These results demonstrate that embryo muscle and heart cells contain the AChE isozymes of their tissues of origin and that innervation is not needed for the appearance of AChE activity in myotubes.

47. Age-dependent Maturation of Embryonic Rat Dermal Collagen in Vitro.[1] K. A. Lindberg and R. L. Hayes (University of Pittsburgh, Pittsburgh, Pennsylvania).

Maturation of collagen fibers is effected by the elaboration of intermolecular cross-links and is manifested through decreased extractability of collagen by inorganic and organic solvents. Our investigation has been undertaken to describe such maturation in embryonic and newborn rat skin cultivated in vitro. Littermates were excised from pregnant rats following 16, 18, or 20 days of gestation. Others were sacrificed 24 hr postpartum (22 days). Dorsal skin explants were maintained as organ cultures over Trowell's T-8 medium supplemented with 10% calf serum and 1.0 μCi/ml of ^{3}H-5-proline. After exposure for 24 and 48 hr, explants were harvested and soluble collagens were extracted. These fractions were assayed for protein-bound ^{3}H-hydroxyproline. Results indicate that collagen synthesis is maximal in 18-day tissue. After 24 hr in vitro, 18-day tissue has relatively less low salt-extracted collagen, indicating a significantly faster turnover into less soluble fractions. At all ages, the low and high salt-soluble collagens are approximately equivalent, suggesting that this maturation occurs spontaneously. The insoluble fraction increases slowly between 16 and 20 days, followed by a rapid increase to represent 60% of the collagen in newborn skin. Hence, dermal collagen maturation in vitro is distinguishable from synthesis and varies with age at explantation.

48. Enhancement of Maturation in Neonatal Dermal Collagen by Copper-Protein in Vitro.[1] R. L. Hayes, K. A. Lindberg, and D. Eberz (University of Pittsburgh, Pittsburgh, Pennsylvania).

In animals deprived of dietary copper, maturation of fibrous collagen is retarded. Presumably, copper is required by enzymes mediating the formation of intermolecular cross-links in collagen fibers. Progressive elaboration of these cross-links is reflected in decreased solubility of collagen and is expressed as maturation of this fibrous protein. Our investigation has been undertaken to demonstrate an influence of copper upon dermal collagen in vitro. Newborn rat skin explants were maintained as organ cultures over nutritive media containing no copper, unbound copper, or copper complexed to liver protein. Our basal medium was no. 199 supplemented with 10% calf serum and ascorbate. Copper sulfate was added to 2.5 μg per ml. Copper-protein medium was prepared by preincubating copper medium with homologous liver slices. After 24 to 48 hr of cultivation, collagen was extracted from the tissues. Soluble and insoluble fractions were assayed for protein-bound hydroxyproline. During exposure to copper-protein, the insoluble collagen fraction increases 20% over the comparable fractions of untreated or unbound copper-treated tissues. Analysis of serum-free copper-protein medium reveals one predominant protein component. This protein has a molecular weight over 150,000, contains less than 1% copper by weight, and migrates electrophoretically as an α-globulin. Our data indicate that protein-bound copper exerts a direct influence upon collagen maturation in vitro.

49. Suppression of Malignancy and Pigmentation in Melanotic Melanoma Cells. S. Silagi (Cornell University Medical College, New York, New York).

A study was made of the effect of 5-bromodeoxyuridine (BUdR) on a melanotic mouse melanoma which grows in vitro with virtually every cell pigmented. These cells can be made to lose all pigment-producing ability within 1 week of treatment with 1 μg per ml and 3 μg per ml of BUdR. The rate of growth is virtually unaffected by these concentrations. The morphology of the cells is altered, with the cells growing in a flattened and somewhat fibroblastic monolayer, with contact inhibition, instead of the rounded, multilayered mounds characteristic of the line. These effects are all reversible. The inclusion of thymidine with BUdR prevents their occurrence. Several cloned

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[1] Supported by Health Research Services and Kozmetsky Foundations.

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BUdR lines have been grown which are capable of indefinite growth in vitro in BUdR with altered morphology. Tumorigenicity is markedly reduced. Untreated melanoma cells produce progressively growing tumors within 3 weeks in 100% of C57BL mice injected subcutaneously with 10^6 cells. No tumors grew within 11 weeks in 30 mice injected with three clones isolated in 3 μg/ml BUdR. Clones isolated in 1 and 2 μg per ml of BUdR had reduced tumorigenicity. To test the required length of pretreatment with 3 μg per ml of BUdR before modification of tumorigenicity occurred, cells were treated from 0 to 21 days in vitro before injection. Cells pretreated for more than 10 days were not tumorigenic. If pretreated for fewer than 10 days, they had reduced tumorigenicity. These results indicate that BUdR, a thymidine analogue which is incorporated into DNA, simultaneously modifies a differentiated function and malignant potential in these cells.

50. Notochord-affected Increase in Cell Number during Vertebral Cartilage Induction in Vitro. DALE W. MATHESON (Department of Anatomy, Milton S. Hershey Medical Center, Hershey, Pennsylvania 17033).

Vertebral cartilage induction, in organ culture as well as in vivo, requires an interaction between competent somites and either spinal cord or notochord. Eight somites (stage 16) cultured with a short segment of notochord formed cartilage in 3 to 4 days. Identical somites without notochord did not form cartilage even after 30 days. A second difference between somite-notochord cultures (SNC) and somites cultured alone (SC) was shown by counting trypsin-dissociated cells in a hemocytometer. Over a 10-day period, the number of cells within SNC increased 5-fold, whereas in SC it remained constant. The possibility that the notochord effected this increase in cell number by stimulating cells to enter the growth fraction was ruled out because autoradiographs of ^{3}H-thymidine-labeled cultures showed that virtually all cells except muscle and older chondrocytes in SNC and muscle in SC were in the proliferative pool. Increased population could also be achieved if the notochord rescued cells from death. To test this idea, loss of ^{3}H-thymidine from a cohort of pulse-labeled cells was monitored daily for 10 days. The results show that more of the original population were retained in SNC than in SC: 97% versus 77% by day 2, 63% versus 37% by day 5, and 30% versus 10% by day 10. Therefore, the notochord clearly augments cell number during induction by prolonging survival of some portion of the total population. The exact constituents of this retained population are unknown, but should prove interesting in terms of cell lineages, cell interactions, and ultimate fates, and the possible role of cell selection during induction.

51. Effect of Bovine Follicular Fluid on the Maturation of Mouse Oocytes in Vitro. I. M. MILLER, A. HAIDRI, AND R. B. L. GWATKIN (Merck Institute for Therapeutic Research, Rahway, New Jersey).

Oocyte arrest at the dictyate stage of meiosis in the adult ovary may be due to one or more factors present in the follicle. We have explored this possibility by placing mouse oocytes with intact germinal vesicles in drops of bovine follicular fluid collected from follicles at various stages of development. After 18 hr in fluid from small follicles (diameter 6 mm or less) only 5% of the oocytes extruded a first polar body, 27% failed to do so and 68% degenerated. In the fluid of large follicles (diameter 7 mm or more) 17% underwent such maturation, 66% failed to do so and only 17% degenerated. Thus, as follicles enlarge, the capacity of their secretions to support oocyte maintenance and development increases. Addition of sodium pyruvate (0.25 mM) or progesterone (0.1 mM) to the inhibitory fluids removed the inhibition. Progesterone did not permit oocyte maturation in Krebs-Ringer-albumin medium lacking an energy source or with glucose, fructose, glucose-6-phosphate, fructose-1,6-diphosphate, glyceraldehyde, phosphoenolypyruvate, 3-phosphoglycerate, lactate, citrate, cis-aconitate, isocitrate, α-ketoglutarate, succinate, fumarate, or malate, nor did it reduce the level of pyruvate which was required for oocyte maturation. These observations are consistent with the hypothesis that secretion of progesterone late in follicle development overcomes diapause of the oocyte by allowing it to utilize some energy source as yet unidentified but not pyruvate.

52. Factors Influencing HeLa Cell Colonial Morphology. JOHN F. FOLEY AND BYRON T. AFTONOMOS (Department of Medicine, University of Nebraska College of Medicine, Omaha, Nebraska).

The authors have previously reported (1967) that HeLa cell colonies grown from single cells may be classified into three types: (1) diffuse—consisting of very loosely scattered cells, (2) intermediate—in which the cells in the center of the colony have become opposed to each other forming a continuous core while the edges still contain loosely arranged cells, and (3) compact—containing cells tightly packed together with frequent formation of multilayers. The following were investigated as their influence on colonial morphology: (a) attachment surface, (b) sera, (c) proteolytic enzymes, (d) highly charged compounds, and (e) other cells. Experiments were done by placing 500 HeLa cells in a modified Eagle's medium containing 20% serum in a 60-mm Petri dish and growing them for 11 days at 36.5°C in a humidified atmosphere of 2.5% CO_2 in air. They were stained and 100 colonies from each of five control and experimental dishes were classified as to type. Glass as contrast to plastic surfaces tend to increase intermediate and compact colonies. Human serum increases diffuse colonies whereas calf and horse sera increase compact colonies confirming the work of Murphy and associates (1962). Crude trypsin and chymotrypsin as well as the charged com-

pounds Darvan no. 1 and heparin increase compact colonies whereas pronase tends to increase loose colonies. In addition, different strains of human fibroblasts increase compact or diffuse colonies when grown with the HeLa cells. Possible explanation for these effects will be presented.

53. Ultrastructure of Choriocarcinoma Line BeWo (Pattillo-Gey). J. E. Rash, M. K. Gey, and G. O. Gey (Johns Hopkins Medical School, Baltimore, Maryland).

Gonadotropin-secreting human choriocarcinoma (BeWo) growing in cell culture almost 4 years was fixed in glutaraldehyde, postfixed in osmium tetroxide and aqueous uranyl acetate. Good preservation of membranous and fibrous components was achieved. En bloc uranyl acetate staining removed the glycogen otherwise electron-dense in sections poststained with uranyl acetate and lead citrate. Glycogen removal produced large clear areas of mottled texture, facilitating the search for other less densely staining components. Abundant cytoplasmic filaments and desmosomes with attached filaments, characteristic of epithelia, were observed. Numerous annulate lamellae were found. This reflected the relatively undifferentiated state evidently continued from oocyte cytoplasm through chorionic development and continuous culture. No particles were observed which were considered to be viruses although many nuclei were observed to possess aggregates of densely staining 500 Å particles, twice the size of ribosomes, which were not associated with nucleoli or nuclear membranes. The high proportion of nuclear cross-sections with one or two aggregates (8%) indicates the presence of at least one aggregate per nucleus. The aggregates persist during mitosis, apparently do not migrate during anaphase, and presumably are not reincorporated into the daughter nuclei. Presently, no function is ascribed to these particles since we have observed similar particles in early embryonic chick cells. The above observations will be compared with normal human chorion.

54. Effect of Ca^{++} and Mg^{++} on Morphology and Growth Pattern of L-M Cells. Da-Ping Yang and Helen J. Morton (Division of Biology, National Research Council of Canada, Ottawa 7, Canada).

Mouse fibroblasts (strain L-M) have been grown in medium M150 plus 0.5% peptone, free of either added Ca^{++} or Mg^{++} for more than 6 months. The cells grew at similar rates to those in complete medium, but with different morphology and growth patterns. The omission of Mg^{++} caused no alteration of cell morphology. The cells were spindle-shaped and well spread, and developed a confluent layer in 4 to 5 days after subculture. In contrast, cells grown in low calcium medium were mostly rounded and grew in clumps. Ball-like aggregates were formed 7 to 10 days after subculturing. These growth patterns were completely reversed when the medium was changed to normal Ca^{++} conc (1.25 mM). Viable staining showed that most cells in an aggregate were alive. On subculturing in complete M150, the aggregates gradually spread, eventually forming a confluent layer. In contrast, aggregates subcultured into fresh low Ca^{++} medium grew out only to a limited extent. Karyotype analysis of the two types of cells revealed no difference; both had a modal number of 65 and showed the same marker chromosomes. This, plus the reversibility of the change by addition or deletion of Ca^{++}, indicates that no population selection was involved. A possible change of cell surface properties resulting from the long term growth of cells in low Ca^{++} medium, and its effect on cell-cell adhesion, will also be discussed.

55. Contributed Paper Session: Oncology, Immunology, Virology and Mitosis. *Chairmen:* K. K. Sanford and R. Ting.

56. Loss of Transplantability by Mouse Ascites Cell Cultures. C. P. Eng and J. F. Morgan (Saskatchewan Research Unit of the National Cancer Institute of Canada, University of Saskatchewan, Saskatoon, Canada).

Inoculation of mice with 100 fully virulent Ehrlich, Ehrlich-Lettré, TA^3, or $6C^3HED$ ascites cells invariably induces 100% tumors and death of the host animals. After prolonged cultivation in vitro, cell lines established from these ascites tumors failed to produce tumors when 10^8 cells were inoculated, either intraperitoneally or subcutaneously, in normal or X-irradiated mice. Further transfer of cells after implantation in X-irradiated mice failed to produce tumors in normal mice. The nontransplantable cells remained viable in the mouse peritoneal cavity for 6 to 8 days and then degenerated. After five washings with Hanks' solution, the nontransplantable cells induced antibody production in rabbits against the calf serum component of the culture medium. After 10 washings, no antibody formation was induced to the calf serum and the cells were found to be still nontransplantable. Loss of tumorigenicity occurred on a different time scale with each ascites tumor cultivated. End point dilution and inoculation of mice at each culture passage have been carried out to investigate the mechanism of the phenomenon.

57. Methylation of tRNA in Neoplastic and Nonneoplastic Mouse Cell Lines. C. E. Quinn, R. Gantt, and V. J. Evans (Tissue Culture Section, Laboratory of Biology, National Cancer Institute, Bethesda, Maryland).

It has been proposed that aberrant methylation of nucleic acids may be a factor in neoplastic conversion. Studies showing an increased tRNA methylase capacity in tumor tissue as compared with that in adjacent normal tissue and quantitative and qualitative differences in the methylated base composition of tRNA from neoplastic tissue tend to support this hypothesis. In the present work an initial attempt has been made to determine what if any changes take place in tRNA methylation in cells which have undergone neo-

plastic conversion and which also demonstrate an increased tRNA methylase capacity. ^{3}H-Methyl-methionine and ^{14}C-methyl-methionine have been added to the growth medium to permit separate labeling and simultaneous extraction, isolation, and chromatography of the tRNA from paired neoplastic and nonneoplastic mouse cell lines. Isotope ratios were calculated from reversed phase chromatography profiles and a consistently reproducible variation in the ratio was seen through the region where the first tRNA's were eluted. Although the observed difference is small, the nature of the controls and the reproducibility of results in repeated experiments, including one in which the radioactive labels were reversed and one using a second paired neoplastic cell line, indicate that this difference is significant.

58. The Influence of Inhibitors of Nucleic Acid Synthesis on Cell Survival and Binding of 7,12-Dimethylbenz[a]anthracene (DMBA-^{3}H). L. Riechers, D. Connell, and J. A. DiPaolo (Biology Branch, National Cancer Institute, Bethesda, Maryland).

The present investigation is concerned with the alteration by nucleic acid inhibitors of the time course of DMBA binding to cells derived from Syrian hamster embryos. In addition, quantitative radioautographic results of the effect of inhibitors on DNA and RNA synthesis are reported. Cultures continuously incorporated DMBA-^{3}H into nuclei during a 7-hr exposure. The rate at which nuclei incorporated the label was affected by the growth rate (doubling time) that was characteristic of the culture. DMBA inhibited growth by 20% at 72 hr. The individual inhibitors, selected (with the exception of 2-mercapto-1-(β-4-pyridethyl) benzimidazole [MPB]) on the basis of cell survival rather than maximum inhibitory effect, caused a 40 to 50% decrease in cell number relative to control cultures. Combinations of carcinogen and inhibitor resulted in the same range of growth inhibition. The DNA inhibitors, hydroxyurea and excess thymidine, had no effect on DMBA-^{3}H incorporation. Excess thymidine was a complete inhibitor of thymidine-^{3}H uptake and an incomplete inhibitor of uridine-^{3}H uptake. Hydroxyurea partially inhibited thymidine-^{3}H uptake. Inhibitors of RNA synthesis, actinomycin D (actD) and MPB decreased incorporation of DMBA-^{3}H. ActD may act as a toxic agent in altering DMBA binding since the concentrations employed lethally damaged more than 60% of the population as indicated by reduction in the percentage of DNA-synthesizing cells 24 hr after treatment although only partial inhibition of uridine-^{3}H uptake occurred.

59. Immunogenic Changes in Tumors Induced by Organ Culture Explantation.[1] B. B. Jacobs (American Medical Center at Denver, Colorado 80214).

A variety of strain specific mouse tumors have been altered immunologically by short term organ culture maintenance in synthetic media. Some of these tumors become serially transplantable in allogeneic hosts across strong *H-2* barriers. The nature of the induction of the change in vitro and of the tumor-allograft interaction in vivo was investigated, using BALB/c functional testicular interstitial cell tumors and DBA/1 recipients. The change is phenotypic since it always is reversed by a single passage of the tumor back through the strain to which it was native. Tumors altered in vitro exert an immunosuppressive effect on their allogeneic hosts. Animals allografted with an unaltered tumor 1 week after inoculation with the same tumor previously altered in vitro will sometimes accept both tumors, whereas allografts of the unaltered tumor alone are always rejected by untreated animals. The reversibility of the alteration as well as the observation that the altered tumors do not grow in 100% of their allogeneic hosts even after 40 serial passages as allografts argues against the role of a selective survival of an antigenically simplified cell population. Selective destruction in vitro of hypothetically more antigenic stromal lymphoid cells is a second possibility which has been investigated. We will present evidence which cumulatively argues against this mechanism. Physiological alteration of the explant in vitro as well as allograft adaptation in vivo play important roles in this phenomenon. We consider that the explanted tissues may produce a state of immunological tolerance when implanted into allogeneic recipients.

60. Cellular Variants for Blood Group H in an Established Cell Line.[1] W. J. Kuhns and S. Bramson (New York University School of Medicine, New York, New York).

HeLa cells are known to possess group H activity, and because of individual cell differences in antigenic reactivity can be separated into subpopulations by specific agglutination with anti-H (ulex). Three populations derived this way exhibited the following characteristics upon staining of clones and subclones with fluorescent-labeled antibody: (a) H^{+} cells, (b) H^{-} cells, (c) H^{+} and H^{-} cells. Metabolic studies were carried out by incorporating ^{14}C-fucose, the immunodeterminant sugar for group H, into growing suspension cultures and separating H^{-} from H^{+} cells by agglutination after 4 days incubation at 37°C. Washed H^{+} cells exhibited 2 times the amount of ^{14}C calculated per cell as did H^{-} cells. The H^{+} population was also richer in mitotic cells as demonstrated by staining techniques. These and long term growth studies suggested that H^{+} cells possessed a selective advantage over H^{-} cells. The distribution of ^{14}C-fucose and group H activity in HeLa cell populations suggest their use as structural markers and present the possibility of using ^{14}C-fucose in con-

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junction with labeled antibody to determine quantity and localization of active blood group sites, relative to total fucose incorporated in and on the cell or cellular variants during their different growth phases.

61. Virus Susceptibility of Cell Lines Derived from Two Species of Marine Mammals. J. T. Cecil and R. F. Nigrelli (Osborn Laboratories of Marine Sciences, Brooklyn, New York 11224).
The susceptibility of five cell lines derived from the heart, lung, kidney, and mesentery of *Halichoerus grypus* (gray seal) and an adrenal cell line from *Lagenorhynchus obliquidens* (Pacific striped or white sided dolphin) were studied for cytopathic changes and their ability to support viral replication. Coxsackie A9 and B1 through B6, reovirus 1 and 3, adenovirus 12, and herpes simplex were inoculated in all cultures with the exception of the dolphin line which was not tested with herpes. The dolphin line was susceptible to Coxsackie A9, B3, and B4 and reovirus 1 and 3, but not Coxsackie B1, B2, B5, B6, and adenovirus 12. The gray seal lung line proved highly susceptible to all viruses tested. The kidney and mesentery cell lines were not susceptible to Coxsackie A9, but all other viruses produced CPE in the cultures. The heart cell line was susceptible to all viruses tested, but only with more concentrated viral preparations. All cell lines were fibroblast-like, with the exception of the gray seal kidney from which both epithelial-like and fibroblastlike cell lines were derived.

62. Investigation on the Toxicity of Polyethylene Glycol in Cell Culture and Its Effect on Virus Growth. M. R. Escobar, H. P. Dalton, and M. J. Allison (Medical College of Virginia, Commonwealth University, Richmond, Virginia).
The purpose of the following experiments was to determine the effect of polyethylene glycol (PEG) of molecular weights 4,000, 6,000, and 20 M on monolayers of H. Ep-2, Rhesus monkey kidney, WI-38, and African green monkey kidney cells. Representative viruses, including polio type 1, adeno type 3, SV_{40}, vaccinia, ECHO type 16, and Coxsackie B type 5 were diluted for titration in solutions of PEG varying in concentration from 4 to 45% for PEG 4,000 and 6,000, and from 4 to 35% for PEG 20 M. Virus-free inocula of these concentrations were also tested on all cells as controls. Results revealed no detectable toxicity of PEG for the cells listed above and no apparent reduction in the titer of the viruses examined. The implication of these findings with regard to the concentration of viruses by hydroextraction will be discussed.

63. Effects of ^{3}H-Thymidine on Mitotic Rate and Chromosomes of Amnion and HeLa Cells. H. C. Wang and S. Fedoroff (Department of Anatomy, University of Saskatchewan, Canada).
The effects of low dosages of ^{3}H-thymidine on the mitotic rate and chromosomes of human amnion and HeLa cells were investigated. The cells were treated with four dosages of ^{3}H-thymidine (0.125 μCi per ml, 0.25 μCi per ml, 0.50 μCi per ml, and 0.75 μCi per ml). After either pulse or continuous treatment, cells were harvested at different time intervals (2 to 17.5 hr) and analyzed. The results indicated that low doses of ^{3}H-thymidine could induce mitotic inhibition and chromatid aberrations. By removing the ^{3}H-thymidine from the medium, the mitotic rate could be restored. The chromosomes were affected only during the S phase, the cells in the early S phase being the most sensitive. The chromatid aberrations did not seem to be related to the duration of the exposure to the incorporated ^{3}H-thymidine but rather to the state of the chromosomes at the time of the treatment with ^{3}H-thymidine. The chromatid aberrations were nonrandom; some chromosomes and even some regions of individual chromosomes were affected more frequently than others.

64. Radiosensitivity of Haploid Cells in Rat Kangaroo Corneal Endothelium Cultures. Kenneth T. S. Yao (Bureau of Radiological Health, Rockville, Maryland).
In our serially cultivated rat kangaroo corneal endothelial cells (42 passages of subcultivation), a number of mitotic cells appeared to have a haploid number of chromosomes. Since the cell culture was derived from the tissue of a female animal, all of the haploid cells had the same number and the same complement of chromosomes—five autosomes and one sex chromosome. These cell cultures were given 0, 20, 50, 100, or 150 rads of X-rays, and the cultures were fixed for making slides at 6, 12, 24, 36, or 48 hr after exposure. Twenty-two to 42% of the mitotic cells were haploid. The percentage of haploid cells increased with the age of the culture and with the X-ray dose. There was a highly significant but negative correlation coefficient (−0.7013) between the percentages of haploids in mitotic cells and mitotic indices. However, the number of X-ray damaged cells was 3 times greater in diploids than in haploids. The average number of chromosome breaks per cell was 0.295 in diploid cells and 0.086 in haploid cells. The average number of breaks per cell per rad ranged from 0.0045 to 0.0142 in diploid cells and from 0.0009 to 0.0046 in haploid cells. The haploids appeared to be much less radiosensitive than diploid cells.

65. Laser Microirradiation of the Juxtanucleolar Region of Prophase Nucleolar Chromosomes.[1] Y. Ohnuki and M. Berns (Pasadena Foundation for Medical Research, Pasadena, California 91101).
To study the function of the nucleolar organizer, we irradiated the region of the secondary constriction in salamander lung cell chromosomes photosensitized with acridine orange, using an argon laser microbeam (Berns et al. Exp. Cell Res., *56:* 292,

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1969; *57:* 60, 1970). Irradiation of this region in anaphase or metaphase chromosomes caused a reduction in nucleolar number in daughter nuclei. To understand further the process of nucleolar formation and its relationship with the cell division cycle, we extend our irradiation to prophase chromosomes. The juxtanucleolar region of nucleolar chromosomes was irradiated and the cell was traced for several days. Nuclei with two nucleoli were generally chosen for irradiation because of their clear set of secondary constrictions. Results are summarized: (a) When either one or several juxtanucleolar sites of all nucleoli are irradiated, they become pyknotic and the mitotic process is blocked. The cells return to interphase and survive up to 10 days. (b) When nucleolar chromosomes of one nucleolus are irradiated, if the irradiated nucleolus is smaller than the unirradiated one, the cells go into metaphase and complete division resulting in a reduction in the number of nucleoli. When juxtanucleolar zone of the larger nucleoli are irradiated, mitosis is blocked. (c) When nucleoli themselves are irradiated, daughter nuclei, however, show no reduction in nucleolar number. (d) The nuclei in which chromosomes are irradiated at the nonjuxtanucleolar regions divide but produce the same number of nucleoli in both daughter nuclei as were present in the mother cell.

66. Chromosomes of the Murine Leukemia Virus Indicator Cell Line XC. W. A. Nelson-Rees (University of California, Naval Biological Laboratory, Oakland, California).

Cell line XC originating from a Rous sarcoma virus-induced rat tumor (Svoboda et al. Folia Biol. (Praha), *9:* 77, 1963) was recently utilized in experiments involving mixed culture cytopathogenicity for the detection of murine leukemia viruses in cell cultures (Klement et al. Proc. Nat. Acad. Sci. U. S. A., *63:* 753, 1969). Although earlier karyologic data were collected on solid tumors and after cultivation in vitro, no such studies were carried out on the cells prior to their utilization for the leukemia virus studies. Presently, the cell line reveals considerable heterogeneity in number and kind in the metaphases sampled, with kind appearing more varied than number. Most metaphases were hypodiploid with reference to the normal rat karyotype (*Rattus norvegicus*, $2n = 42$), all had one to several chromosome(s) nontypical of the rat, some had aberrant chromosomes (dicentric, tricentric), but all conformed to the rat karyotype by several criteria. Neither stemline nor karyotype stability within a modal number of chromosomes was established. These studies are preliminary to future assay studies utilizing clonal derivatives.

67. Some Effects of DMBA on Embryonic Mouse Skin in Organ Culture.[1] A. Singh and Margaret H. Hardy (Department of Biomedical Sciences, University of Guelph, Guelph, Canada).

In a comparison of the effects of hormones and carcinogens on embryonic skin in organ culture, 1 μg per ml of 7,12-dimethylbenz(a)anthracene (DMBA) was added to the medium (cock plasma and chicken embryo extract in the ratio 3:1). Explants of skin from the trunk of 13.5 to 15-day mouse embryos were grown in Maximow single coverslip assemblies for 3 to 6 days. Living explants in control medium showed normal development of hair follicles, but, after only 1 day in DMBA medium, follicles had failed to elongate and made little progress subsequently. Control explants showed progressive stratification of the epidermis with a sharply demarcated basement membrane, and a stratum corneum after 2 days, but all these features were less distinct or absent from treated explants. Sections stained with hematoxylin and eosin from control explants at 3 days showed normal histological differentiation, but treated explants revealed a disordered dermis, regressing follicles, enlargement of cells, nuclei, and nucleoli in stratum spinosum, and marked parakeratosis with absence of typical cornified cells. After 6 days in vitro most of these differences were accentuated. In addition, the basement membrane appeared discontinuous in PAS-stained sections, and stratification of the epidermis had disappeared from some areas. Some of these effects are similar to those reported to occur much more slowly following local application of carcinogenic hydrocarbons to skin of intact mice. However, the coverslip method enables the exact sequence of events to be observed microscopically in living tissues.

[1] Supported by grants from National Research Council of Canada (A4278) and National Cancer Institute, Canada.

68. Invited Symposium: Cell Cycles and Synchronized Cell Populations. *Symposium Chairman and Organizer:* R. M. Nardone (Catholic University of America). (To be presented in three sessions.)

69. Invited Symposium: Session I.—Synchronized Cell Populations and Their Use as Experimental Tools. *Chairman:* F. H. Kasten (Louisiana State University).

70. Natural and Induced Synchronous Cultures. G. F. Whitmore (Ontario Cancer Institute).

71. Parasynchrony and Synchrony in Animal Cells, in Situ. E. Mattingly (University of Georgia).

72. Synchronous Cultures in Cytodifferentiation Studies. R. J. Neff (Vanderbilt University).

73. Curbstone Conferences, *organized by:* J. E. Shannon, Jr. (American Type Culture Collection, Rockville, Maryland). (To be presented in four sessions.)

74. Curbstone Conference: Session A.—Com-

mittee on Sera. *Chairman:* A. J. KNIAZEFF (University of California, San Diego).

75. Curbstone Conference: Session B.—Committee on Chemically Defined Media. *Chairman:* H. J. MORTON (National Research Council, Ottowa, Canada).

76. Curbstone Conference: Session C.—Committee on Contaminations in Cell and Tissue Cultures. *Chairman:* J. FOGH (Sloan-Kettering Institute for Cancer Research, Rye, New York).

77. Curbstone Conference: Session D.—Committee on Tissue Culture Materials and Supplies. *Chairman:* J. E. SHANNON, JR. (American Type Culture Collection, Rockville, Maryland).

78. Round Table Discussion: Cell Culture in Space Biology. *Organized and chaired by:* J. F. SAUNDERS (National Aeronautics and Space Administration, Washington, D. C.).

79. Round Table Discussion: Cell Culture in Space Biology. P. MONTGOMERY (Southwestern Medical School, Dallas, Texas).

80. Round Table Discussion: Cell Culture in Space Biology. C. R. FISHER (Manned Space Center, Houston, Texas).

81. Session in Depth: Plant Tumors, Their Origin, Genetic Constitution, and Growth. *Chairmen:* D. DOUGALL (Ohio State University, Columbus, Ohio) AND T. STONIER (Manhatten College, Bronx, New York).

82. Action of Viral DNA on Tobacco Stem Tissue. JUDITH LEFF AND ROBERT BEARDSLEY (Manhattan College, Bronx, New York 10471).

Previous experiments have shown that the DNA extracted from a bacteriophage propagated on a sensitive strain of *Agrobacterium tumefaciens* could induce tumors on stems of sunflower plants and primary leaves of pinto bean plants. The present experiments were undertaken to test phage DNA under conditions where the possibility of accidental contamination by *A. tumefaciens* would be obviated. The phage, designated PS8, was originally isolated from tissue derived from a crown gall tumor on a sunflower stem. DNA was extracted from phage particles harvested from lysates of infected bacteria and applied to tissues of internode segments of tobacco (*Nicotiana tabacum* L. var. Havana) in vitro. The internode segments were cultured on the medium of Murashige and Skoog without auxin or kinetin. After 3 days in culture, the cut surfaces of the segments were punctured with a needle and the various preparations to be tested were applied with a pipet. DNA caused the formation of tumor-like proliferations whose growth was strictly dependent on the concentration used. Experiments to determine whether or not the DNA-induced growth has the characteristics of tumorous growth are currently in progress.

83. Mechanism of Tumor Formation in Plants by *Agrobacterium tumefaciens*.[1] B. I. SAHAI SRIVASTAVA (Roswell Park Memorial Institute, Buffalo, New York).

Whether transformation of a normal plant cell to tumor cell by *A. tumefaciens* was brought about by changes in the normal cell chromatin or by incorporation of an "infective genome" into host genome was examined. No significant differences in the T_m, in vitro RNA-synthesizing activity, or the composition (DNA-histone-residual protein) of chromatin from normal and tumor tissue cultures of tobacco were noted and the ratios of F_{I}:F_{II}:F_{III} histones from the two tissues varied only slightly. On the other hand, RNA-DNA hybridization studies revealed the presence of a RNA fraction in the tumor tissue which hybridized with the *A. tumefaciens* DNA. This RNA fraction was not detected in the normal tissue. These results and others bearing on the crown gall problem will be presented.

84. Studies on the Antigenic Composition of the Crown Gall Tumor System. DENES DE TOROK AND ROBERT A. CORNESKY (Carnegie-Mellon University, Pittsburgh, Pennsylvania).

Since the crown-gall disease is initiated by an uncharacterized tumor-inducing principle (TIP), that is elaborated by a specific bacterium, *Agrobacterium tumefaciens,* being consistently involved with the transformation of a non-self-limiting neoplastic disease of plants in at least 142 genera, belonging to 61 genetically widely differing botanical families, it was desirable to characterize the antigenic interrelationships of these systems. For the localization and characterization of the various specific antigens, disc electrophoresis and agar immunoelectrophoresis of extracts from different strains of *A. tumefaciens,* including some of which fail to induce recognizable tumors; primary tumors; bacteria-free secondary tumors, grown in vitro; and uninfected extracts of all the plants studied were employed, using rabbit antisera. Cross-absorption studies of the various rabbit antisera with homologous and heterologous plant and agrobacterial extracts showed certain antigens that were not only unique to each of the above but also displayed partial antigenic determinants within the groups. The antigenic cross-reactivity of tumors induced by the same system, the antigenic differences between different groups of tumors, and the antigenic individuality induced by the same parameters imply a certain relationship between antigenic pattern and etiology.

85. Plant Tumor Induction by Wound Tumor

[1] Supported by United States Atomic Energy Commission Contract AT(30-1)37221.

Virus. G. STREISSLE AND H. A. WOOD (Boyce Thompson Institute, Yonkers, New York).

The wound tumor virus (WTV) is an insect-borne plant tumor virus which contains a double stranded ribonucleic acid (ds RNA) genome of high molecular weight. Like other RNA tumor viruses WTV causes no cytolytic effect and is readily detected in tumors. However, the virus apparently disappears from wound tumor tissue cultivated in vitro. Despite the loss of virus, the tissue culture maintains its abnormal growth. In view of the possibility that naked viral nucleic acid may still be present in apparently virus-free wound tumor cells and may be responsible for their tumor character, a characterization of WTV RNA was undertaken. The RNA extracted from purified virions was fractionated on sucrose gradients and by polyacrylamide gel electrophoresis. The RNA segments occurred in eight different size classes with sedimentation coefficients ranging from 10 to 15 S. All eight classes consisted of ds RNA. A random fragmentation of WTV RNA appeared unlikely since the size and ratio of the RNA classes remained unchanged under all conditions tested. When WTV RNA was isolated directly from the plant tumors, the ds RNA classes found were identical to those obtained from purified virions; however, a different molar ratio was observed. The results of attempts to isolate viral RNA from apparently virus-free wound tumor tissue cultures suggested the presence of ds RNA.

86. Heritable Tumor Formation in Tobacco. G. L. HAGEN (The Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania).

Heritable tumor formation in some tobacco hybrids is a function of the proper intermixing of two unique genomes. The chromosomal involvement in this tumorogenic response and the informational translation from these chromosomes in terms of physiology and biochemistry are being analyzed. Various hybrids and hybrid derivatives are produced by hand pollination. These hybrids are studied cytologically and morphologically to determine chromosomes present and the tumor incidence. These plants are then studied both nutritionally (i.e., tissue culture of stem and pith) and biochemically (i.e., analysis of hormones, enzymes, and hormonal function) and compared on the basis of their known genetic background. The results show that only a minimal parental genome is involved in tumorogenesis which results in an elevated in vivo auxin (hormone) level and a lack of requirement for the hormone in vitro. Both the synthesis of the hormone and the oxidative ability to control its level appear to change in tumor potential tissue. The elevated auxin level results in an apparent increase in RNA and protein synthesis in the tissue. Thus, the major modification that occurs in some tobacco hybrids is tumor growth, and this tumor growth appears to be directly related to elevated hormone levels which translates into cells capable of both rapid cell enlargement and cell division, neither one of which is fundamentally controllable by the cells.

87. Transport of ^{14}C-IAA in Tumorous and Nontumorous Nicotiana Stem Tissue.[1] M. H. BAYER (The Institute for Cancer Research, Philadelphia, Pennsylvania 19111).

Tumor formation in plants has been associated with increased auxin levels in these tissues. To test whether this hypothesis holds for tumor-producing *Nicotiana* hybrids versus the nontumorous parental species *N. glauca* and *N. langsdorffii,* an investigation of diffusable auxin of stem tissues of these plants was carried out (Amer. J. Bot., *51:* 546, 1964). Hybrid stem tissue showed a decreased amount of diffusable auxin as compared to its parents, whereas chromatographic analyses of tissue extracts yielded a higher amount of indoleacetic acid and other indole auxins in tumor-prone hybrid plants. It was suggested that the reduction of diffusable auxin from hybrids might be due to a decreased transport capacity for auxin in these tissues (Plant Physiol., *44:* 267, 1969). Therefore, to study the capacity for transporting auxin in parental and hybrid tissues, measurements were made on the flux and velocity of ^{14}C-IAA in stem sections. Measurements of transported labeled IAA were made after 15, 20, 30, 45, 60, and 120 min through 6-mm long sections of third internodes. The sections were cut into 1-mm slices and the activity of ^{14}C-IAA in these slices was counted on a Packard liquid scintillation counter. A decreased transport capacity in tumor-prone hybrid tissue as compared to that in nontumorous parental tissues could be observed. The reduced capacity for auxin transport in the hybrids before the onset of tumorogenesis might be one of several factors involved in the production of spontaneous tumors on these plants.

88. Session in Depth: Insect Cell Lines, Their Origin, Identification, and Characterization. *Chairman:* M. STANLEY (University of Virginia, Fairfax, Virginia).

89. Leafhopper Cells: Morphology and Growth Potentialities in Primary Cultures and Cell Lines. H. HIRUMI (Boyce Thompson Institute, Yonkers, New York).

Some plant pathogens are transmitted by Leafhopper vectors and paurometabolus insects and multiply in both insect and plant cells. Leafhopper cell culture was developed during the past decade with the aim of studying interrelationships between the plant pathogens and vector cells in vitro. The first breakthrough in the development of techniques came in 1964, in our laboratory, when the superiority of embryonic tissues, particularly in the blastokinetic stage, for providing active cell growth was discovered. Since then embryonic cells

[1] Supported by U. S. Public Health Service Grants CA-04890, CA-06927, and FR-05539 from the National Institutes of Health, and by an appropriation from the Commonwealth of Pennsylvania.

from 10 different species have been successfully cultured in several laboratories. Four types of fibroblast-like cells, five types of epithelial cells, one type of hemocytes, and three other cell types have been described. In all cases, three types of cells became dominant. One type was represented by fibroblast-like cells with long, dipolar pseudopodia forming networks, the second had large epithelial cells with numerous large cytoplasmic granules, and the third was composed of smaller epithelial cells devoid of granules. The epithelial cells grew from tissue fragments of embryonic brains, legs, thoraxes, abdomens, digestive tracts, etc., and formed compact sheets of cells. The second major breakthrough was achieved by Chiu and Black in 1967, and resulted in the establishment of Leafhopper cell lines consisting of heterogeneous cell populations from five different species. One of these, *Agallia constricta* cell line-2 (AC2), was composed of epithelial cells. The morphological resemblance between the AC2 cells and the epithelial cells, usually dominating in the primary cultures, was striking. Leafhopper embryonic cell cultures, as well as cell lines, are mixed populations of male and female cells.

90. Characteristics of Some Insect Cell Lines. W. F. Hink and B. J. Ellis (The Ohio State University, Columbus, Ohio).

Five established in vitro cultured insect cell lines were investigated to compare growth rate, chromosome complement, cellular DNA content, and morphology. The lines are Grace's *Antheraea eucalypti* and *Aedes aegypti* and Hink's *Trichoplusia ni* (TN-368), *Carpocapsa pomonella* (CP-1268), and *C. pomonella* (CP-169). Line TN-368 grows most rapidly with a population doubling time of 16 hr and a lag phase of less than 24 hr. CP-1268 and CP-169 have population doubling times of 24 hr with lag phases of 24 and 72 hr, respectively. *A. aegypti* and *A. eucalypti* population doubling times are 42 to 48 hr with 24-hr lag phases. Maximum populations per milliliter of media are 1 to 2 $\times$ 10^6 for TN-368, *A. aegypti* and *A. eucalypti*, 5 $\times$ 10^6 for CP-169, and 10^7 for CP-1268. All lines are polyploid and frequency distributions of chromosome numbers provide characteristics for distinguishing specific lines. CP-1268 chromosome distribution is bimodal with 49% of cells containing from 52 to 57 chromosomes and 33% with 102 to 108 chromosomes. The CP-169 line differs in that 8% of cells contain 52 to 57 chromosomes. Most of cells of line TN-368 contain 82 to 95 chromosomes. DNA content is another distinguishing characteristic. The DNA content per 10^6 cells is 19.4 μg for *A. eucalypti*, 13.7 μg for *A. aegypti*, 9.0 μg for CP-169, 6.3 μg for CP-1268, and 6.0 μg for TN-368. All lines contain fibroblast-like and spherical shaped cells with significant differences in cell size and percentage of cell types. TN-368 fibroblast-like cells are approximately twice as long as those of other lines. The spherical cells of TN-368, CP-1268, and CP-169 are smaller than those of *A. aegypti* and *A. eucalypti*. CP-169 contains fewer spindle-shaped cells and more spherical cells than other lines.

91. Techniques for the Preservation and Characterization of Insect Cell Cultures. A. E. Greene, J. C. Charney, and L. L. Coriell (Institute for Medical Research, Camden, New Jersey).

The development of insect cell cultures offers cell biologists new approaches to the study of various animal and plant diseases. For this reason we have attempted to cultivate a number of insect cell lines for inclusion in the National Reference Cell Bank stored at the ATCC. The cell characterization consists of developing techniques, for freezing away in liquid nitrogen large batches of ampules containing at least 1,000,000 cells per ampule. Thawed ampules are then characterized by chromosome analysis, isoenzyme patterns, serologic species identification, and virus susceptibility. Isoenzyme analysis utilizing a battery of different isoenzymes and agar immunodiffusion are reliable methods for detection of contamination with cultures of different species. Although insect cell culture presents some methodology differing from mammalian cell culture, the characterization for certification for the National Reference Cell Bank parallels in most instances techniques utilized in mammalian cell characterization.

92. Metabolism of *Aedes aegypti* Cells Grown in Vitro. 1. Incorporation of ^{3}H-Uridine and ^{14}C-Leucine. Y. Hayashi and S. S. Sohi (Insect Pathology Research Institute, Canada Department of Fisheries and Forestry, Sault Ste. Marie, Ontario, Canada).

The objective of the experiments was to study the metabolic activity of cell cultures of the mosquito *Aedes aegypti*. Such information would be of much practical use in determining the nutritional requirements of these and other insect cell cultures, and for studying other factors affecting their growth in vitro. Radioactive tracer technique in conjunction with a liquid scintillation spectrometer was used in these investigations. The work was restricted to the incorporation of ^{3}H-uridine and ^{14}C-leucine. "Chase" experiments with unlabeled precursors, and the use of actinomycin D and puromycin, showed that ^{3}H-uridine was incorporated into cellular RNA, and that ^{14}C-leucine was incorporated into protein of these cells. Incorporation of ^{3}H-uridine was inhibited when actinomycin D was used at a concentration of 10 μg per ml, and ^{14}C-leucine incorporation was inhibited to the same extent by puromycin at a concentration of 100 μg per ml of medium. These experiments indicated that the *A. aegypti* cell cultures have a high metabolic activity.

93. Initiation and Characterization of Insect Cell Cultures. Janiece S. McHale, E. Cupp, H. D. Unthank, and B. H. Sweet (Gulf South Research Institute and Tulane University, New Orleans, Louisiana).

Primary cultures from three orders of insects have been initiated. Various organs of both sexes (*Diptera*) as well as larvae (*Coleoptera*) and hemolymph from dipausing pupae (*Lepidoptera, Coleoptera*) were employed. Media (Grace's, Singh's, and Leafhopper) used to initiate the cultures affected cellular morphology and growth. Mosquito lines, *Aedes vexans* and *Culiseta inornata,* previously initiated in our laboratory as mixed populations of suspended cells have been carried in Grace's medium without hemolymph for over 2 years. The cultures had been split weekly at a ratio of 1:10 until recently, when an increase in cell doubling time necessitated a 1:15 weekly split ratio. Optimal growth occurred at 28°C, but the cultures were induced to adapt to 31°C by low split ratios over a period of several weeks. Thereafter, the cultures increased in doubling time until a 1:10 split ratio was required weekly. These high temperature variants were cultured again at 28°C, but retained their characteristic growth rate at the lower temperature, distinguishing them from the parent lines. *A. vexans* and *C. inornata* cells are highly polyploid and morphologically similar to Grace's moth line, *Antheraea eucalypti.* Some spindle-shaped cells closely resemble Suitor's clone of Grace's *Aedes aegypti* (MSQ-68). All of these suspended cell lines may represent hemocytes originally adherent to tissue explants. Some differences, however, were discerned among these cultures by their responses to different growth media. Antigenic interrelationships among various insect lines and larvae are the subject of another paper from our laboratory.

94. Antigenic Interrelationships between Mosquito Cell Culture Lines and Mosquito Fourth Instar Larvae as Shown by Immunodiffusion Methods. Adly N. Ibrahim, E. W. Cupp, and B. H. Sweet (Gulf South Research Institute, and Tulane University, New Orleans, Louisiana).

Investigations were undertaken to study the antigenic interrelationships between mosquito cell lines and mosquito fourth instar larvae by the immunodiffusion method. Hyperimmune rabbit sera prepared against each cell line were reacted with their corresponding antigen preparation as well as with antigens prepared from the whole extracted larvae. Similarly, rabbit antilarvae sera were tested against the mosquito cell lines. The results may be summarized as follows. (a) Each antiserum reacted with its homologous antigen. (b) Antisera prepared against the monolayer cultures of Singh's or Peleg's lines derived from *A. aegypti* and/or *A. albopictus* cross-reacted with each other. (c) Antisera against the suspended lines of *A. aegypti* failed to cross-react with the monolayer lines of *A. aegypti.* (d) *Antheraea* antiserum reacted broadly with most cell culture antigens (both suspended and monolayer), but not with whole larval antigens. (e) Antisera against mosquito larvae did not react with any insect cell lines. (f) Antisera prepared from all mosquito cell lines reacted broadly with larval antigens of *A. aegypti, A. taeniorhynchus, A. albopictus, C. pipiens pipiens, C. pipiens fatigans,* and *A. stephensi.* These results indicate the complexity of the problem with which one is faced in distinguishing insect lines from each other or from the whole organism from which they were derived. Further studies are needed before immunological "markers" for insect cell lines can be established.

95. Vitamin B_{12}-dependent Insect Cell Line. J. C. Landureau (Faculty of Science, Paris, France).

The recent progress being made in the cultivation of cockroaches' embryonic cells has provided the possibility, not realizable until the last years, of studying the nutritional requirements of insect cells for amino acids and hydrosoluble vitamins. We have currently developed synthetic media without either serum or any protein supplement; the cell protection ensured by the serum antiproteases could be supplied by 0.5% of Ficoll (mol wt 400,000). In such a way, the growth of an established insect cell strain (EPa strain) appeared to be dependent upon vitamin B_{12} and afforded an outstanding opportunity to investigate biosynthesis and metabolic pathways.

97. Contributed Paper Session: Hormones and Membranes. *Chairmen:* R. A. Pattillo and R. B. L. Gwatkin.

98. Human Growth Hormone in Cultures of Human Pituitary Tumors. U. Batzdorf, V. Gold, N. Mathews, and J. Brown (University of California at Los Angeles School of Medicine, Los Angeles, California).

Growth hormone (GH) immunoassays have been conducted on 14 cultured human pituitary tumors by analyzing the culture medium at different periods of growth. GH was identified in significant quantity in the culture medium of tumors from all six patients who clinically showed evidence of acromegaly. It was absent from all but two of the culture media of tumors derived from eight hypopituitary patients. One of the two exceptions represented the culture medium from a tumor removed during pregnancy. GH was also identified in the culture medium of pituitary tissue removed from a diabetic patient. The close correlation between clinical state and significant quantity of GH in the culture medium helped to confirm the diagnosis of acromegaly.

99. Action of Certain Hormones on Cells Growing Continuously in Chemically Defined Media. C. T. Ling and G. O. Gey (Johns Hopkins Medical School, Baltimore, Maryland).

Hormones help to regulate the life processes of cells, thus enabling the whole organism to attain normal growth and development, ecological adaptation, immune responses, and the maintenance of homeostasis. Serum or tissue extracts used in cell cultures contain various hormones in varying concentrations. Cell cultures growing continuously in

chemically defined media (7C's AN-54) without supplement of serum or tissue extract have been used for culture studies of the action of hormones, singularly or in combination. Although some of the hormones are species specific, bovine somatotropin is active for rat cells in culture. Other studies involve the effect of thyroxine and of corticosteroids. Walker 256 rat carcinosarcoma cells growing for 2 years in 7C's media showed a stimulation of growth when somatotropin was added enabling them to spread out as single cells and to improve the general cell morphology. In one experiment with W-256, bovine somatotropin (activity, 1 unit per ml) added at 50 mg per ml gave almost double the average cell protein per tube compared to those cultures without the hormone. Levothyroxine used singly did not have a significant growth-promoting effect on MBIII mouse lymphoblasts or W-256 cells under similar conditions. In another experiment, cortisol was found to stimulate the growth of Chang's liver cells, line L-39, also growing in 7C's media. Detailed data will be presented.

100. Androgenic Response in Target Tissues in Organ Culture with Differentiating Gonads.[1] Y. S. Moon, Margaret H. Hardy, and J. I. Raeside (Department of Biomedical Sciences, University of Guelph, Guelph, Canada).

Several lines of evidence suggest that the testis of the domestic pig secretes androgen during early fetal life. In order to investigate this, portions of the androgen-sensitive ventral prostate gland of the 21- to 28-day rat were grown in organ culture beside entire gonads from fetal pigs of 20-mm crown rump length, or portions of gonads from 25- to 30-mm fetal pigs. Prior to cultivation, the prostate glands showed small thick walled acini lined by cuboidal or low columnar epithelium and containing very little secretory material. After 6 days in medium TC199 + 10% calf serum, isolated explants of prostate gland remained healthy, and showed slight regression of the epithelium and little change elsewhere. Testes from 25-mm and 30-mm pig fetuses developed normally in vitro, and adjacent prostate gland segments responded with a secretory epithelium, enlarged acini containing some secretion in the lumen and reduction in the stroma. Ovaries from similar pig fetuses did not affect adjacent prostate explants. The indifferent gonads from 20-mm pig fetuses which differentiated into testes after 6 days in vitro stimulated prostate explants in the same way but those which subsequently differentiated into ovaries caused no change in the rodent glands. It was concluded that the observed changes were due to androgens which are secreted by the male gonad even before sex can be determined histologically. Histochemical evidence presented elsewhere supports this conclusion and strongly suggests that the androgens in question are steroids.

101. Hormonal Effects on Enzymes of a Cell Line Derived from Normal Rat Liver. L. E. Gerschenson (University of California, Los Angeles, California).

A technique to dissociate normal rat liver and further culture the cells has been developed. The cells have been cultured continuously for over 9 months. Studies performed with the electron microscope showed some characteristics similar to hepatocytes. The organ function specificity of the cells has been evaluated by measuring typical liver enzymes as tyrosine α-ketoglutarate transaminase, glucokinase, alcohol dehydrogenase, etc., and the effect of insulin, dexamethasone, and estradiol-17β upon them.

102. Hydroxysteroid Dehydrogenase Activity of Rat Ovarian Follicular Cells in Vitro. T. V. Fischer and R. H. Kahn (University of Michigan, Ann Arbor, Michigan 48104).

Ovarian follicular cells from the mare and pig, cultivated as monolayers, secrete progesterone for several weeks. This study investigates the ability of the more available rat follicular cells to produce progesterone in vitro. Isolated ovarian follicular cells from both ovaries were pooled and incubated for 6 days in Leighton tubes. Trowell's T-8 medium plus 10% fetal calf serum was changed as required and saved for progesterone assay. Cells maintained as a monolayer rapidly developed large coalescing vacuoles which are stainable for lipid (oil red O). Assuming that the lipid may represent synthesized steroid, the histochemical procedure for hydroxysteroid dehydrogenase activity was utilized to determine whether progesterone can be produced in vitro by rat follicular cells. The coverslips were incubated for 1 hr in 0.2 M phosphate buffer (pH 7.5) containing 2 mg of DPN, 0.5 mg of nitro BT, and 0.25 mg of dehydroisoandrosterone (substrate) per ml. Control incubations lacked substrate. Specimens were then fixed and counterstained. Activity of Δ^5-3β-hydroxysteroid dehydrogenase was evident within all cells (except controls) as a fine blue granular deposition. Since this enzyme is necessary for the conversion of pregnenolone to progesterone, these results indicate that the rat follicular cell cultures are able to synthesize this hormone.

103. Hormonal Requirements to Develop Lobuloalveolar System in Mouse Mammary Glands. Kamal J. Ranadive, T. N. Chapekar, and S. V. Gadkari (Cancer Research Institute, Bombay, India).

Newly developed inbred strain of albino mouse designated ICRC strain is susceptible to spontaneous breast cancer and leukemia. Multiaspect in vivo and in vitro studies have been carried out on this strain in comparison with the high tumor strain C3H. Experimental data are already published indicating significant basic differences in the hormonal factor of these two strains. In view of these findings, the present studies were undertaken to determine hormonal requirements for development of lobuloalveolar system in the two strains C3H and ICRC. Mammary glands from

[1] Supported by Grant MA3566, Medical Research Council of Canada.

5- to 8-week old virgins and early and late prelactating mothers of strains C3H, ICRC, and cancer-resistant strain C57BL were organotypically cultured by a modified watch glass method developed by Chapekar. Cultures were incubated at 34–35°C for 9 to 15 days and treated with various ovarian and pituitary hormonal combinations, differing in quantity and quality, to find out specific hormonal milieux necessary for differentiation of the alveolar system in the mammary glands. These experiments clearly elucidated that the hormonal requirements of the three strains were different and specific. Hormonal requirements of ICRC virgin mammary glands to develop lobuloalveoli were prolactin 3 times and hydrocortisone 2 times more than that required by the C3H glands. Early prelactating mammary tissue of the ICRC mice also required increased level of the hormones to differentiate lobuloalveolar system and initiate secretion.

104. Aggregation and Growth Behavior of Cultured Chinese Hamster Cells: Relationship to Drug Resistance and Oncogenic Potential.[1] J. L. Biedler and B. A. Spengler (Sloan-Kettering Institute, New York, New York).

A variety of Chinese hamster cell lines sensitive and resistant to actinomycin D, daunomycin, amethopterin, and 5-bromodeoxyuridine (BUdR) showed characteristic patterns when trypsinized cells were allowed to aggregate on a gyratory shaker for 24 hr. Sensitive parental cells (DC-3F and CLM-7), as well as amethopterin and BUdR-resistant strains, formed loose clusters whereas antibiotic-resistant cells formed dense aggregates according to degree of resistance. Similarly, patterns of growth on glass substrate fell into two groups, with either disoriented and discrete or oriented and compact cell distribution. Previous studies indicated that resistance to actinomycin D and daunomycin was due to alterations of surface membrane resulting in decreased permeability to drug, while resistance to amethopterin and BUdR was due primarily to changes in target enzyme activities. Furthermore, parental lines and the several amethopterin-resistant DC-3F sublines tested were tumor-producing in Syrian hamster cheek pouch, whereas heterotransplantability of the antibiotic-resistant sublines was reduced or abolished according to degree of resistance. Observations of these cell lines thus suggest that membrane differences in the actinomycin D- and daunomycin-resistant cells result in decreased drug sensitivity, decreased tumor-producing capacity, and increased cellular adhesiveness in vitro.

105. The Potassium Inhibition Threshold of Individual Pacemaker Cells Isolated from the Embryonic Chick Heart. R. L. DeHaan and E. W. Schaefer, Jr. (Carnegie Institute of Washington, Department of Embryology, Baltimore, Maryland).

High levels of extracellular potassium (K_0) tend to depress excitability and spontaneous activity of heart tissue. Cells dissociated from hearts of 7-day chick embryos, cultured at 1 to 2 $\times$ 10^6 cells per plate, such that 80 to 90% make no contact with any neighbor, manifest this sensitivity to K_0 quantitatively. In a medium containing 1.3 mM K, 45 to 50% of these single isolated cells were found to beat spontaneously. Replicate cultures in medium differing only in that they contained 4, 8, or 12 mM of K_0 exhibited respectively about 25%, 12%, and 6% beating cells (DeHaan. Develop. Biol., *16:* 216, 1967; DeHaan and Gottlieb. J. Gen. Physiol., *52:* 643, 1968). To determine whether each cell has a reproducible K-inhibition threshold, fields of cells were photographed by phase optics on Polaroid film, while maintained under optimal environmental conditions in a microscope stage incubator. K_0 was increased stepwise to 3.8, 7.0, or 9.6 mM, or in a single step to 8.4 mM, by injecting small aliquots of 40 mM KCl. After each increment, the cells which had stopped beating were identified on the photograph. The plate was then washed twice in 1 mM K medium and reequilibrated for 2 hr, and the incremental series of K injections was repeated. Out of a total of 476 individually identified cells so treated, an average of 84.9% of the original beating cells stopped at the same level of K_0 both times. We conclude that most pacemaker cells derived from the 7-day embryonic heart exhibit individual thresholds for K inhibition which remain constant for at least several hours in culture.

106. ATP Effect on Membrane and Alkaline Phosphatase Activity of HeLa and HEp-2 Cells. E. Miedema (Southern State College, Springfield, South Dakota).

Regulation of alkaline phosphatase, a membranous enzyme in cultured human cells, has been found to be influenced by population density and cell contact phenomena. Inference was made of a role for alkaline phosphatase in the migratory activity of cells. In the present study it was of interest to know whether ATP could cause changes in HeLa and HEp-2 cell membranes and/or modify the alkaline phosphatase activity of the cells. Since prednisolone has been reported to alter the membrane properties of cultured cells, its effect in this study involving ATP was also considered. Incubation of intact human cells grown in vitro with ATP (0.04 to 2.00 mg per ml) for 1.25 to 2.50 min resulted in 37 to 100% inhibition of alkaline phosphatase activity determined by histochemical and chemical assays. ATP added to cell-free lysates did not inhibit alkaline phosphatase activity. After 5- to 20-min incubation with ATP, the cells exhibited increasing resistance to deoxycholate lysis, trypsin, and Pronase action, which suggested that their membrane properties had been altered by the trinucleotide. Results show that ATP has rapid and dramatic effects on cell membranes (HeLa and HEp-2 lines) as measured by increased resistance to lysis by deoxycholic acid and inhibition of alkaline phosphatase activity. These changes were apparent within 5-min exposure of

[1] Supported by NCI Grant CA 08748.

intact monolayer cells to ATP, but the trinucleotide had no effect on alkaline phosphatase activity if added to the cultures after cell lysis by deoxycholic acid. (Work done at the Samuel Roberts Noble Foundation, Inc., Ardmore, Oklahoma 73401.)

107. Properties of Mucosubstances Isolated from the Cell Periphery of Normal and Malignant Cells.[1] J. BEIERLE, Y. TAKAHASHI, L. BAVETTA, AND S. ALLERTON (University of Southern California, Los Angeles, California).

Mucosubstances at the cell periphery are suggested to play a role in numerous cell-cell interactions. Utilizing EDTA in isotonic solutions to disperse cells, we have isolated such substances from the periphery of normal BHK and polyoma virus-transformed hamster kidney cells (PY), and from a malignant human kidney cell, the Wilm's tumor. These cell-free isolates were comparatively examined by physical and chemical techniques, and they were also assayed for any effect that they may exert on cell proliferation. In all samples examined, no glycoproteins were detected as determined by the absence of sialic acid and fucose, whereas proteins, amino sugars, and uronic acid could be detected in all of the preparations. RNA, but not DNA, was also detected in the PY and BHK preparations. The isolates were all of a relatively low molecular weight (less than 100,000) and physical examination of the components by column chromatography and analytical ultracentrifugation revealed no gross differences between normal and malignant cell extracts. Fractions obtained from the PY and Wilm's cells were found to promote cell proliferation in vitro, whereas no such effect was noted when analogous fractions from normal cells were assayed. These extracts are also immunologically distinct from any media component.

108. The Effects of Phthalthrin Insecticide on the Ultrastructure of Cell Membranes. D. R. BRANSON AND P. H. CLEVELAND (The Dow Chemical Company, Midland, Michigan 48640).

Cell membranes have been suggested to be the site of action of some insecticidal chemicals. Evidence for this suggestion was by action potential relaxation time of nerve cells exposed to a pyrethroid insecticide (Berteaú, Casida, and Narahashi. Science, *161:* 1151, 1968). Our study was concerned with possible ultrastructural lesions in cell membranes due to insecticide insult. A synthetic pyrethroid, phthalthrin (±*cis,trans*-isomer), was applied to the growth media of primary rabbit kidney cells, 70 to 90% monolayer, for 2 hr. The cell surfaces were examined with the aid of transmission and scanning electron microscopes. A loss of microvilli and the appearance of microgranules around the cytoplasmic membrane were the earliest dose related responses. These responses were not found with insecticides of other chemical families, i.e., chlorinated hydrocarbons, carbamates, or organic phosphates, at any concentration. These results demonstrate new methods for studying cell membranes and further implies a possible target for insecticide synthesis.

[1] Supported by U S Public Health Service [1] PO1 DE 02848-01.

109. Cytophagocytosis by Human Tumor Cells in Vitro.[1] HARRY L. IOACHIM AND MARLENE SABBATH (Dept. of Pathology, Columbia University, College of Physicians and Surgeons and Lenox Hill Hospital, New York).

Cytophagocytosis is not restricted to specialized cells and tumor cells frequently display a capacity to engulf erythrocytes, lymphocytes, and even tumor cells of their own kind. The latter is observed both in histological sections and in tissue cultures of human tumors. To study this phenomenon, a standard HEp2 culture was used and various attempts made to induce cytophagocytosis. Serum or lymphocytes of rats injected repeatedly with HEp2 cells were added to HEp2 monolayers and cytophagocytosis was recorded. Subsequently, suspensions of HEp2 cells from such cultures presumably specifically modified were added to monolayers of untreated HEp2 cells. In other experiments, different types of cell alterations were induced in HEp2 cultures by ultraviolet irradiation and various antimetabolites. Electron microscopy was used to observe cellular engulfment and ^{3}H-thymidine to label and identify HEp2 cells added to HEp2 cultures. Cellular aggregation, adhesion, and engulfment were more frequent with altered HEp2 cells. Degradation of phagocytosed cells resulting in intracytoplasmic but not intravacuolar inclusion bodies was common. Polykaryotic cells as a result of fusion were also more numerous. It is postulated that cytophagocytosis is a cell membrane phenomenon related to recognition and degradation of altered cells with subsequent reutilization of cellular material.

110. Possible Determinants of the Strain Specificity of Fusion from Without by Newcastle Disease Virus (NDV). W. R. GALLAHER AND M. A. BRATT (Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts).

We have previously reported that fusion of chick embryo fibroblasts by NDV is of two types. Fusion from without (FFWO) is the direct result of a virion-cell membrane interaction, while fusion from within (FFWI) is the result of successful, productive infection. These phenomena are both strain-specific and largely mutually exclusive, such that a given strain may be a good inducer of FFWI, yet produce virions incapable of FFWO (and vice versa).[1] Studies on factors affecting FFWO and examination of other NDV-specific cell membrane interactions suggest that this strain specificity may result from an antagonism between the fusion process and the oppositely directed

[1] Supported by Grant CA10273-03 from the National Cancer Institute.

[1] *In Vitro,* 1970, in press.

phenomena of elution and penetration. (a) We find that there is a tendency for good inducers of FFWO to elute slowly from red blood cells, while poor inducers of FFWO tend to elute rapidly. (b) We have reported that, after adsorption at 4°C, FFWO occurs rapidly at 54°C but not at all at 23°C.[1] We have since found that, if cultures are initially shifted to 23°C for as little as 10 to 20 min prior to further incubation at 43°C, FFWO is irreversibly inhibited. Possible mechanisms of this inhibition include elution and penetration since both processes occur at 23°C. We conclude that the initial virion-cell membrane interaction is a prime determinant of fusion from without by NDV.

111. Scientific Demonstrations, *organized and chaired by:* J. E. SHANNON, JR. (American Type Culture Collection, Rockville, Maryland).

112. Demonstration of Tissue Typing Techniques. K. W. SELL (National Naval Medical Center, Bethesda, Maryland).

113. Demonstration of Perfusion Techniques for Maintaining Mammalian Organs. T. MALININ, G. MALININ, V. P. PERRY, S. WOODARD, AND T. MOUER (Biomedical Research Institute, Rockville, Maryland).

114. Insect Tissue Culture Technique. M. STANLEY (George Mason College, Fairfax, Virginia) AND J. VAUGHN (National Naval Medical Center, Bethesda, Maryland).

115. Plant Tissue and Organ Culture Techniques. G. SCHAEFFER AND J. ROMBERGER (U. S. Department of Agriculture).

116. Invited Symposium: Session II. The Cell Cycle. *Chairman:* E. V. GAFFNEY, II (Pennsylvania State University, Hershey, Pennsylvania).

117. Programmed Synthesis of DNA during the Cell Cycle. J. H. TAYLOR (Florida State University).

118. Enzyme Induction and the Control of Gene Expression during the Cell Cycle. G. TOMPKINS (University of California Medical Center, San Francisco, California).

119. On the Mechanism of Mitosis: the Role of Iron. E. ROBBINS (Albert Einstein College of Medicine).

120. Session in Depth: Cell Membrane Alterations. *Chairman:* V. C. MCKHANN (University of Minnesota, Minneapolis, Minnesota).

121. SV-40 Genetic Function in Transformed Cells. M. N. OXMAN, M. J. LEVIN, A. S. LEVINE, AND P. H. HENRY (Childrens Hospital Medical Center and Harvard Medical School, Boston, Massachusetts, National Cancer Institute, Bethesda, Maryland, and University of Missouri School of Medicine).

Simian virus 40 is capable of initiating the oncogenic transformation of cells in vivo and in vitro. Such transformation involves a number of heritable alterations in cell phenotype, some of which appear to be virus-specific. The correlation of cell phenotype with the persistence and function of SV-40 genetic information will be considered, and the nature of the relationship between viral and cellular DNA in transformed cells will be discussed.

122. A General Change in Membrane Structure of Mammalian Cells after Viral or Chemical Transformation. V. K. JANSONS AND M. M. BURGER (Department of Biochemical Sciences, Princeton University, Princeton, New Jersey).

Various agglutinins have been found to react with transformed cells either exclusively or to a greater extent than with their normal counterpart, indicating qualitative or, in some cases, quantitative differences in the surface structure. The receptor site of one agglutinin was isolated and partially purified and turned out to be present also in normal cells but apparently not available to the agglutinin on the intact cell. Brief exposure to low concentrations of proteases reversibly exposed as many receptor sites in normal cells as did transformation by any virus studied. Even though the agglutinin receptor site is not exclusively cancer-specific and may be partially exposed in some normal cells, the transformation process is always accompanied by an additional exposure similar to that obtained with proteases. A correlation between availability of receptor site and loss of density-dependent inhibition of growth has been observed. Recently we found that low concentrations of proteases trigger division of normal tissue culture cells thereby bringing about an escape from density dependent inhibition of growth. This observation strengthens the suggestion of a relationship between the receptor site and membrane structure changes due to transformation.

123. Carbohydrates of Mammalian Cell Surfaces.[1] M. C. GLICK (University of Pennsylvania, Philadelphia, Pennsylvania).

Surface membranes are the site of the major percentage of some of the carbohydrates representing glycoproteins and glycolipids of mammalian cells. We are studying the relationship of these carbohydrate-containing molecules to oncogenesis by (a) examining the total monosaccharide composition of surface membranes and/or whole cells under a variety of conditions including transformation with DNA and RNA viruses, (b) studying the synthesis and turnover of surface membrane components, and (c) looking directly at the glycoproteins of the cell surface with the use of radioactive precursors (double label techniques). In the

[1] Supported by grants from the U. S. Public Health Service and the American Cancer Society.

latter approach, BHK cells, transformed by two strains of RNA viruses were compared to their normal counterparts after prelabeling with ^{14}C- and ^{3}H fucose or glucosamine. Examination on Sephadex G-200 of the material (TRM) removed from the cell surface with trypsin revealed the transformed cells contained TRM shifted slightly toward higher molecular weight products. Isolated surface membranes showed a similar shift. The shift was not apparent by prelabeling with Leu, Val, and Phe. When TRM was further digested with pronase and examined on Sephadex G-50 it was found that the transformed cells contain fucose-oligosaccharides not present in the normal. Glucosamine incorporation differed in some respects from fucose but again shows the presence of oligosaccharides in TRM from transformed cells which are not seen in the normal. Reverse labeling showed similar results. It is proposed that fucose, a terminal component of glycoproteins, along with sialic acid, could be used for regulation of cell surface events. Other experiments with synchronous populations of KB cells support this concept.

124. Ultrastructure of Cell to Cell Contacts between Malignant Cells.[1] N. S. McNutt and R. S. Weinstein (Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts).
Several lines of evidence indicate that an abnormality at the cell surface may account for the invasive nature of malignant growth. In epithelial malignancies, quantitative deficiencies in cellular cohesion may permit invasion of surrounding tissues. For example, the observed decrease in cohesion of cells in cervical carcinoma (Coman, D. Cancer Res., *4:* 625, 1944) may have its ultrastructural correlate in: (a) decreased area of 200-Å close apposition of adjacent plasma membranes, (b) fewer desmosomes, or (c) a decrease in the number of nexuses. Thus far, a striking decrease in nexuses is the most consistent abnormality reported in carcinomas (McNutt and Weinstein. Science, *165:* 597, 1969) and in certain transformed cells in culture (Martinez-Palomo et al. Cancer Res., *29:* 925, 1969). At nexuses, the adjacent plasma membranes form a specialized region of contact consisting of interlocking globular subunits which permit a type of intercellular communication (McNutt and Weinstein. Biophys. Soc. Abstr., *14:* 101a, 1970). A deficiency of nexuses and a consequent loss in communication between carcinoma cells may result in some of the abnormal growth patterns exhibited by carcinoma cells in vivo and in vitro.

125. Behavior of Fibroblasts on Surfaces. V. M. Ingram (Massachusetts Institute of Technology, Cambridge, Massachusetts).
The adhesion of fibroblasts to inert surfaces and to each other is a property of the cell membrane which is only poorly understood. On the other hand, there are many clear instances where oncogenic transformations are in part expressed as obvious changes in cell surface properties such as adhesiveness and locomotory behavior on solid substrates. In this sense the study of locomotion bears on the understanding of molecular changes in transformed cells. Time lapse microphotography enables us to explore possible mechanisms of locomotion of fibroblasts and of the contact inhibition of locomotion. Side views of normal and sarcoma cells show marked differences. A discussion of the adhesive properties of cell membranes is also relevant.

126. Human Tumor Antigens. D. L. Morton (Tumor Immunology Section, Surgery Branch, National Cancer Institute, Bethesda, Maryland).
Tumor-specific transplantation antigens (TSTA) capable of inducing a host immune response which specifically retards the growth of neoplastic cells have been demonstrated in a wide variety of viral and carcinogen-induced neoplasms, as well as in certain spontaneous animal neoplasms. It is logical to assume that human neoplasms contain TSTA similar to those found in animal tumors, but the tumor transplantation techniques used to demonstrate these antigens cannot be applied to man. However, a variety of sensitive serological techniques have now been used to demonstrate tumor antigens which are immunogenic in the autologous host in a variety of human tumors including Burkitt's lymphoma, malignant melanoma, neuroblastoma, skeletal and soft tissue sarcomas, and colonic neoplasms, as well as other types of human malignancies. This paper will briefly review the antigens associated with various types of human neoplasms, summarize evidence that some of these antigens function as TSTA, and consider the relationship of these antigens to viruses associated with certain types of human neoplasms. The antigenic systems associated with human sarcomas and melanomas will be discussed in detail.

127. Contributed Paper Session: Insect and Plant Culture. *Chairmen:* G. Hagen and W. F. Hink.

128. Studies on Cell Proliferation in Vitro. I. K. Vasil (Department of Botany, University of Florida, Gainesville, Florida).
The following work was initiated in order to follow and understand the significance of changes occurring at the histochemical and ultrastructural level during cell proliferation and callus formation in vitro. Seeds and seedlings of sweet pea (*Lathyrus odoratus*), carrot (*Daucus carota*) root, and parsley (*Petroselinum hortense*) petiole tissues were used. Tissues exposed to 2,4-dichlorophenoxyacetic acid and a variety of other phenoxyacetic acids and plant growth substances for as little as 24 hr significantly alter their course of development and differentiation and undergo extensive cell proliferation in order to form callus tissues.

[1] Supported by Grant CA-07368 from the National Cancer Institute of the National Institutes of Health.

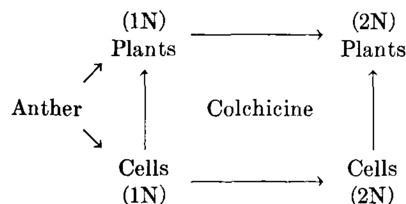
One of the first signs of cell proliferation is increased pyroninophilia (indicating high ribonucleic acid content) of the cells destined to undergo cell divisions. These cells show larger nuclei in relation to their total volume, nonvacuolated cytoplasm, and a high histone content, localized mainly in the nucleus and the chromosomes. At the ultrastructural level proliferating cells are characterized by lobed nuclei and the presence of a very large population of ribosome-like particles around the nucleolus. These particles are thought to be representing the increased synthesis of ribonucleoproteins and ribosomal subunits in response to growth substance treatment and the altered course of development and differentiation. There are significant qualitative differences in amino acid composition and the protein profiles of control and treated tissues. Experiments are in progress now to localize the site of action of the growth substances causing cell proliferation and callus formation by high resolution autoradiography at the electron microscope level.

129. Manipulation and Fusion of Plant Cell Protoplasts.[1] R. U. Schenk and A. C. Hildebrandt (Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin, 53706).

Fusions were achieved of peanut and soybean protoplasts produced with purified commercial cellulases from cells grown in tissue culture. Reduced osmolality (ØSm ≈ 0.40) and lower solution density permitted protoplasts to come into intimate contact and to expand during emergence. These may be important factors inducing fusions. Increased osmolality (ØSm ≈ 1.0) also favored fusions, but the "sticky" membranes adhered tenaciously to any glass surface including micropipets and microcups used in manipulating them. Calcium ion retarded fusions, but perfusion with isotonic, 50 mM EDTA solution caused protoplasts to burst. Pneumatic fluids for micromanipulation of protoplasts were evaluated. Fluorocarbons proved toxic to protoplasts. Further experimentation indicated that aqueous solutions were preferable to organic fluids. Preferred properties of the fluid include: density >1.05, pH ≈ 6.0, ØSm ≈ 0.50, nontoxic to and not absorbed by protoplasts, low to moderate viscosity, and not degraded by microorganisms. Ficoll to increase density and salts to increase osmolality were used, but slow microbial growth was observed. Polyethylene glycols are being evaluated. Protoplasts were manipulated in hanging drops in a moist chamber. Precautions were necessary to restrict moisture loss from the solutions. The ultimate objective of this work is the production of somatically hybridized plants.

130. Growth and Morphogenetic Potencies of Pith and Anther Callus Cultures Derived from Several Varieties of *Lycopersicum*. W. R. Sharp and D. K. Dougall (Ohio State University, Columbus, Ohio).

The objective in the following investigation is to study the feasibility of *Lycopersicum* anther culture in the development of new homozygous plant varieties from which plant lines could be selected for enhanced productivity, greater adaptability, or disease resistance. We are investigating the various routes to such plants. These routes are summarized in the model below:



Although, Nitsch was able to get haploid plants directly from tobacco anthers we have not been able to accomplish this with tomatoes. However, we have succeeded in establishing anther and pith cultures from tomatoes. The growth and morphogenetic protencies of these cultures have been determined using a wide variety of media containing various concentrations of indole acetic acid, 2,4-dichlorophenoxyacetic acid, kinetin, adenine, hydrolyzed casein, and coconut milk. These results will be discussed.

131. Probable Inhibition of Lignification in Sunflower Crown Gall Tissue by Auxin Protectors.[1] T. Stonier (Manhattan College, Bronx, New York 10471).

Auxin protectors are antioxidants that inhibit certain peroxidase catalyzed reactions. Lignin formation is known to involve a peroxidase-catalyzed reaction. Sunflower crown gall tissue lignifies dramatically when transferred to certain "low mineral" media. A study of protector leaching showed that protectors leach out more rapidly into the "low mineral" medium than in "high mineral" medium (in which the tissue grows well and shows relatively little lignification). Adding protector to the low mineral medium did not inhibit lignification, but neither did adding large amounts (3×10^{-4} M) of catechol (a substance which mimics protectors and is probably related to protectors chemically). The externally added catechol became oxidized to its *o*-quinone, a strong oxidant, a fate that also presumably befell the externally added protector. The addition of a strong antioxidant which does not become oxidized to a strong oxidant, viz., dithiothreitol, inhibited lignification. These results indicate that endogenous auxin protectors inhibit lignification and that the protectors act as cellular regulators which determine whether plant cells divide or differentiate.

[1] Supported by Agricultural Research Service, U.S.D.A. Grant 112-14-100-9905 (34). Crops Research Division, Beltsville, Maryland.

[1] Supported by Damon Runyon Memorial Grant DRG 933.

132. Ribosomal RNA Precursor Synthesis in Tobacco Tissue Culture. TSAI-YING CHENG (The Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania).

Phytohormones are known to influence plant nucleic acid metabolism. Numerous studies further indicate that auxins both enhance the synthesis of all RNA species and preferentially increase the synthesis of rRNA when compared to sRNA and mRNA. In this study the aseptic suspension cultures of white friable undifferentiated callus cells such as those derived from pith explants of the tobacco hybrid of *Nicotiana glauca* and *N. langsdorffii* were used to obtain precise data on RNA metabolism. These cells have several unique advantages including: (a) they are free from microorganism contamination which has frequently interfered with plant nucleic acid analysis; (b) they are nonphotosynthetic cells, eliminating confusion from chloroplast metabolism; (c) they are an essentially homogeneous mass of parenchyma cells with meristematic activity; (d) they can be manipulated in a manner comparable to a bacterial system, thus facilitating the experimental scheme; and (e) their uptake of radioactive compounds is rapid and uniform. Further, the techniques of sucrose density gradient sedimentation and polyacrylamide gel electrophoresis were used instead of MAK column chromatography, the latter widely used in plant nucleic acid research. The results show that the phytohormone IAA stimulates RNA synthesis. Analyses by sucrose density gradient sedimentation, polyacrylamide gel electrophoresis and actinomycin D pulse-chase experiments show a rapidly labeled ribosomal RNA precursor with a sedimentation coefficient of 38 S and a molecular weight of 2.8×10^6 daltons using *E. coli* rRNA as a reference. An intermediate component with a molecular weight of 1.5×10^6 daltons is also detected.

133. In Vitro Maintenance, for as Long as 5 Years, of a Cell Differentiation. P. JOLLES AND J. C. LANDUREAU (Faculty of Science, Paris, France).

An insect cell strain from *Periplaneta americana* L. embryos was used in our study. The cells regularly produced in the culture medium a lytic substance able to destroy rapidly several kinds of microorganisms. Biochemical data first established an unusual occurence of chitinase in the metabolized medium: the enzymatic concentration measured against *Micrococcus lysodeikticus* cells was equivalent to that of a 10 to 20 μg per ml solution of hen egg white lysozyme. By electron microscopy, tubular granulations were characterized; the ultrastructure changed in function of time and these changes are related to the quantity of chitinase released in the medium. Some possible relationships between these preliminary results and those previously reported on the insect hemolymph will be discussed.

134. Cytogenetical Analysis of an Established Cell Line of *Drosophila melanogaster*. S. DOLFINI, L. TIEPOLO, AND A. M. COURGEON (Institute of Genetics, Milan, Italy, EURATOM Unit for Human Radiation and Cytogenetics, Pavia, Italy, Laboratoire de Biologie Animale, Paris V, France).

We studied the cell cycle and the karyotypic variation of a cell line (K_C) established in 1969 from primary cultures of embryonic cells by Echalier and Ohanessian. Duration of the phases of the cell cycle was estimated by mean of pulse labeling with tritiated thymidine. The average lengths of G_1, S, and G_2 were 2, 9, and 7 hr, respectively. The major part of the cells were diploid, while between 5 and 10% were tetraploid. Aneuploidy and structural rearrangements were present and consisted mainly of (a) loss of one of the chromosomes of the IV pair; (b) splitting of the Y chromosome into two portions, one acentric presumably translocated onto the heterochromatic segment of the X chromosome and the centric one either present and of variable length or absent; (c) deletion of a segment of one of the two large autosomes. Replication of DNA was studied by autoradiography with special reference to the chronology of replication of heterochromatin.

135. Metabolism of *Aedes aegypti* Cells Grown in Vitro. II. Determination of Cell Viability. S. S. SOHI AND Y. HAYASHI (Insect Pathology Research Institute, Canada Department of Fisheries and Forestry, Sault Ste. Marie, Ontario, Canada).

The objective of the study was to find a method of differentiating between living and dead cells in *Aedes aegypti* cell cultures. We tried the dye exclusion test using erythrosin B as the stain. The dye solution was prepared in basic salt solution (BSS). Three concentrations of the dye were tested: 20 mg per 100 ml of BSS, 40 mg per 100 ml of BSS, and 80 mg per 100 ml of BSS. To each sample of cell suspension an equal volume of dye solution was added. The lowest concentration gave the best results. When cell counts were made at different intervals after adding the dye to cell suspension, there was no significant change in the number of stained cells over a 1-hr period in 20 mg of erythrosin B per 100 ml of BSS, but the number of stained cells increased with the passage of time in higher concentrations of the dye. Leaving the cells at 5°C up to 24 hr prior to addition of dye altered neither cell number nor viability. A close correlation was found between the incorporation of ^{14}C-leucine and the number of viable cells. These experiments indicate that erythrosin B can be used for differentiating between viable and nonviable *A. aegypti* cells.

136. Observations on Cell Cultures in the Class Insecta. W. W. NICHOLS, C. BRADT, AND W. BOWNE (Institute for Medical Research, Camden, New Jersey).

This communication deals with cytogenetic observations on cell cultures derived from three spe-

cies of the class Insecta. These observations were made in the course of characterizations of the cell lines at one of the cooperating laboratories of the Cell Culture Collection Committee. The first line, *Antheraea eucalypti,* has a large number of chromosomes with poor morphology, including many minutes. There is no distinct stemline number and the range of chromosome number extends from 90 to 270. While these cells are a poor subject for chromosomal study, they have very prominent spindle fibers and offer excellent material for studies of mitotic mechanism. The second and third lines are derived from the mosquitoes, *Aedes albopictus* and *Aedes aegypti.* Both of these have a distinct stemline number of 6 and excellent chromosome morphology. These demonstrate an interesting difference, in that *A. aegypti* has maintained its diploid character, while the *A. albopictus* has a pseudodiploid character.

137. Contributed Paper Session: Metabolism. *Chairmen:* V. J. CRISTOFALO AND K. McCARTY.

138. Requirements for Cholesterol, Hematin, and Lecithin for Optimal Growth of a Porcine Kidney Cell Line. KIYOSHI HIGUCHI (Biological Sciences Laboratories, Department of the Army, Fort Detrick, Frederick, Maryland).

The nutritional requirements for growth of a porcine kidney (PK) cell line in a chemically defined medium were studied in cells grown as monolayer cultures in T-30 Falcon Plastic flasks incubated at 36°C with caps tightened. The PK cell appeared to be unique among a variety of heteroploid cell lines in its requirement for a number of unusual substances. Successful propagation of PK cells was obtained in a serum-free defined medium that contained cholesterol (2×10^{-5} M), hematin (0.5 μg per ml), lecithin (2 μg per ml), and coenzyme Q_{10} (1 μg per ml). The PK cell line may serve as a useful tool in a study of intermediary lipid metabolism at the cellular level.

139. Stimulation of DNA Synthesis of Rat Salivary Gland Cells in Monolayer Cultures by Isoproterenol (IPR). JOHN W. KREIDER (College of Medicine, Pennsylvania State University, Hershey, Pennsylvania).

IPR is a potent stimulator of DNA synthesis in the salivary gland parenchymal cells of intact rats. Although the exact mechanism of action has not been shown, it seems most likely that IPR acts directly upon these cells and does not require an intermediary interaction with endocrine glands or the nervous system. To test this hypothesis, monolayer cultures of cells obtained from rat salivary gland cells were established. It is likely that at least some parenchymal cells were originally present in the cultures, for some epithelioid cells were associated with fibrillar extracellular material which reacted like sialomucin with Spicer's histochemical method. No amylase activity was ever detected in the cultures. In repeated experiments, IPR significantly enhanced the uptake of tritiated thymidine into TCA-insoluble cell fractions. Optimal responses were obtained with an IPR dose of 50 μg per ml. Higher doses were inhibitory. The cultures gradually lost responsiveness to the drug with continued passage, concomitant with the slow disappearance of epithelioid cells and their replacement by fibroblast-like cells. It is concluded that IPR can directly stimulate DNA synthesis in salivary gland cells without the intervention of endocrine or neural systems.

140. *Mycoplasma*-induced Tissue Culture Medium Modifications. E. J. STANBRIDGE,[1] F. T. PERKINS, AND L. HAYFLICK (M. R. C. Laboratories, London, England, and Stanford University, California).

Both fermenting and nonfermenting *Mycoplasma* species induce chromosomal aberrations and morphological changes in cultured cells. The present studies were undertaken in order to observe changes in composition of cell-free media in which mycoplasmas were grown that might produce such anomalies in cultured cells. Initial efforts revealed that fresh or conditioned media supplemented with calf serum did not support the growth of any of seven *Mycoplasma* species studied. The limiting factor(s) was found to be a nucleic acid precursor(s). Amino acid analyses were made on cell culture media in which the mycoplasmas had been grown. Results clearly indicated that the only amino acid completely depleted was arginine and that only nonfermenting mycoplasmas depleted the medium of this amino acid. We conclude that the induction of chromosomal aberrations is due to competitive utilization of a nucleic acid precursor(s) and, in the case of nonfermenting mycoplasmas, may also be due to arginine depletion.

141. Utilization of RNA Ribose for Glycolysis in Cultured Mouse Glioblastoma Cells.[1] R. LASHER AND W. KIRSCH (University of Colorado Medical Center, Denver, Colorado).

The glioblastoma, a malignant astrocytic tumor, is poorly vascularized, a condition associated with tumor anerobiosis. Despite poor oxygenation, the tumor is able to survive and grow. Carbon balance studies of glycolysis in these tumors have conclusively demonstrated that degradation of glucosyl residues (glucose, glycogen) is insufficient to account for the amount of lactic acid produced (Kirsch, W. Cancer Res., *25:* 432, 1965). The discovery of purine ribosidase in this tumor has directed attention to a ribose source for generated lactate. To test this hypothesis, whole cell-labeled RNA is extracted from cultured glioblastoma cells previously incubated with uridine-^{3}H (generally labeled) for 24 hr, and then added to the culture medium of unlabeled cells for varying periods of time. The cells are then lyophilized and fraction-

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[1] Present address, Stanford University.

[1] Supported by Grant 69-14 from the Milheim Foundation for Cancer Research and CA-08594, U.S. Public Health Service.

ated in glycerol by a nonaqueous method (Kirsch, W., et al., manuscript in preparation). The labeled acid-soluble fraction in the cells is resolved by thin layer chromatography for determination of labeled lactate. Cultured mouse glioblastoma cells can take up exogenous RNA and metabolize it to lactate in both nucleus and cytoplasm. The data indicate that a significant amount of high energy phosphoryl bonds utilized in metabolic pathways for tumor cell replication and survival is derived from degradation of certain species of tumor RNA.

142. Induced Changes in Glucose Metabolism of HeLa Cells by Prednisolone. P. R. Barnes and G. Melnykovych (U. S. Veterans Administration Hospital, Kansas City, Missouri, and Department of Microbiology, University of Kansas Medical School, Kansas City, Kansas).
Experiments were performed in order to investigate the effects of prednisolone on selected aspects of glucose metabolism in cultured heteroploid cells. HeLa cells of the S3 line were grown as monolayers in Eagle's MEM medium supplemented with 10% calf serum. The cells were exposed to prednisolone at a final concentration of 1×10^{-6} M during different stages of their growth cycle and then examined for glucose consumption, lactic acid production, glycogen content, and incorporation of uniformly labeled ^{14}C-D-glucose into different cellular components. Cells exposed to this hormone for 10 hr exhibited greater glucose consumption and lactic acid production than the steroid-free controls. No significant change in the intracellular glycogen content was observed when the cells were grown in the presence of prednisolone for 24 hr. However, when the cells were maintained under these latter conditions and then pulsed for 30 min with uniformly labeled ^{14}C-glucose, there was 30% less recovery of radioactivity in the lipid, protein, DNA, and RNA cellular fractions. This latter phenomenon induced by prednisolone after 24 hr could reflect either decreased uptake of the substrate or partial inhibition of macromolecular synthesis.

143. Competitive Binding of Steroids in Subcellular Fractions of HeLa Cells. C. F. Bishop and G. Melnykovych (U. S. Veterans Administration Hospital, Kansas City, Missouri, and Department of Microbiology, University of Kansas Medical School. Kansas City, Kansas).
The competitive binding of C_{21} steroids previously reported for intact cells (Melnykovych, G., and C. F. Bishop. Biochim. Biophys. Acta. *177:* 579, 1969) was studied in nuclear, mitochondrial, microsomal, and cytosol fractions prepared from HeLa cells. The cells were pretreated with cortexolone (Reichstein Cpd. S, 11-deoxycortisol), pulsed with cortisol-^{3}H, and fractionated. The results revealed a characteristic temperature-dependent distribution of specific binding among the cell tions. At 0°C the major portion of the specifically bound steroid was localized in the cytosol, whereas at 37°C there was a shift of the labeling from cytosol to the nuclear fraction. The results suggested an energy-dependent translocation of the steroid-receptor complex from the cytosol into the nucleus, resembling a similar process for estradiol binding in rat uteri which had been reported by Jensen and Gorski. Density gradient analysis of cytosol and of the nuclear extract has confirmed the existence of competitive binding among several C_{21} steroids. The importance of the cell membrane in this process was suggested by the observation that the cells partially damaged by removal from glass before labeling showed an increased nonspecific binding of cortisol in the cytosol fraction.

144. Relation between Resistance to 5-Fluorouracil, 5-Fluorodeoxyuridine, and Thymidine in Ehrlich Ascites Carcinoma Cells in Vitro. William J. Reeves, Jr., and Relda M. Cailleau (M. D. Anderson Hospital and Tumor Institute, Houston, Texas).
The relationship between the development of resistance to 5-fluorouracil and the development of cross-resistance to thymidine and 5-fluorodeoxyuridine was studied in an established strain of Ehrlich ascites carcinoma cells which was sensitive to growth inhibition by fluorouracil, fluorodeoxyuridine, and thymidine. Sublines were derived that were 10 to 15 times as resistant to fluorouracil and 8 to 10 times as resistant to thymidine as the parent strain by long term growth in the presence of gradually increasing concentrations of these substances. Chromosome studies showed that the three lines differed in modal number of chromosomes and in number of minutes and/or metacentrics. When tested for cross-resistance, it was found that the fluorouracil-resistant subline was also resistant to fluorodeoxyuridine and thymidine, whereas the thymidine-resistant subline was sensitive to fluorouracil but resistant to fluorodeoxyuridine. These findings suggest that the loss of the salvage pathway for uracil (fluorouracil resistance) is associated with loss of the thymidine kinase pathway (thymidine and fluorodeoxyuridine resistance) but that the reverse is not true.

145. Actinomycin D Effects on Steroidogenesis by Adrenocortical Cells. G. C. Yuan and E. D. Bransome, Jr. (M. I. T., Cambridge, Massachusetts).
For our investigation replicate Petri dish cultures of a cloned line of mouse adrenocortical tumor cells (strain Y-1 donated by G. Sato) were grown in Ham's F-10 medium supplemented with 15% horse and 2.5% fetal calf serum, at 37°C in a humid 5% CO_2-95% air atmosphere. Base line and ACTH-stimulated steroid production rate in cells exposed to 0.001 to 10 μg of Act.D per ml 7 to 10 days after subculturing was unaffected for up to 8 hr. Within 14 to 22 hr, the effect of ACTH on cells exposed to 0.1 μg of Act.D was decreased, but not without a drop in base line steroidogenesis. New medium was restored, cell count, microscopic observation, and steroid production were then followed for up to 2 months. Within 1 day cells density was unaffected but, within 2 to 5 days, considerable cell death,

even after exposure to levels of Act.D which had no apparent effect during the 1st day, and a parallel inhibition of steroidogenesis were observed. These effects were directly related to the amount of and duration of exposure to Act.D. Only within 2 to 5 weeks was there a recovery of cell density, base line steroidogenesis, and ACTH response (the latter sometimes exceeded control levels). Act.D effects on steroidogenesis are thus linked with severe, often irreversible cell damage which is not immediately apparent. With adrenocortical cells in culture (and perhaps other cells also) Act.D inhibition of cell function may indeed be related to cell death.

146. Control Mechanisms of L-Asparagine Biosynthesis in Mammalian Tissues. M. K. PATTERSON, JR., G. O. ORR, AND E. CONWAY (The Noble Foundation, Inc., Ardmore, Oklahoma).
The sensitivity of malignant tissues to L-asparaginase (ASNase) has been related to the levels of cellular L-asparagine synthetase (ASN SYN) activity. Normal tissues having low levels of the latter enzyme have shown increased enzyme activity following ASNase treatment. Similar adaptive changes were found in malignant cells resistant to ASNase but not in cells sensitive to the drug. Inhibitors of protein synthesis prevented the adaptive change. An in vitro model system which mimics the in vivo normal tissue changes has been used to study further the mechanism of control of asparagine synthesis. Cells fully adapted to ASNase, as evidenced by high ASN SYN levels, show a 2- to 3-fold decrease in enzyme activity following the addition of L-asparagine. The results suggest a "repression-derepression" control mechanism of L-asparagine synthesis on ASNase-resistant and normal tissues.

147. Association of Aminoacyl Transferase II with Ribosomes of HeLa Cells during Amino Acid Deprivation. M. E. SMULSON (Georgetown University, Washington, D.C.).
Aminoacyl transferase II (T-2) is the enzyme involved in the translocation reaction of mammalian polypeptide chain elongation. It is established that diphtheria toxin inhibits protein synthesis of susceptible mammalian cells by inactivation of T-2. We found that residual protein synthesis of HeLa cells deprived of a single essential amino acid was resistant to diphtheria toxin. Association of T-2 with ribosomes has been shown to protect against toxin inactivation, and we found that there was a 2-fold increase in ribosome-bound T-2 in intact cells starved for an amino acid. Most of the enzyme existed in unbound form in the cytoplasm of cells; surprisingly, very little was found associated with either heavy or light polyribosomes in control or starved cells; the 2-fold increase, noted above, was reflected in binding to the monoribosome fractions. Cells incubated in Eagle's medium in the presence or absence of tyrosine contained approximately the same amounts of free 60 S and 40 S ribosomal subunits. Amino acid-starved cells were mainly characterized by increased pools of free 74 S ribosomes. In both experimental conditions, we found T-2 association only with the 74 S ribosomes and not with the ribosomal subunits. This agrees with recent in vitro data. T-2, accumulated on 74 S ribosomes during amino acid starvation of intact HeLa cells, was rapidly released by readdition of the deprived amino acid and restoration of protein synthesis. The data suggest cyclization of T-2 in intact cells.

148. Special Library Session: Methods of Indexing and Titling to Ensure Best Use of Tissue Culture Literature. H. CESVET (Librarian, W. Alton Jones Cell Science Center, Lake Placid, New York).

149. Special Hormone Session. Tissue Culture for Hormones in Vivo. *Chairman:* L. J. LEWIS (Abbott Laboratories, North Chicago, Illinois).

150. Tissue Culture for Hormones in Vivo. A. STEINBERGER (Albert Einstein Medical Center, Philadelphia, Pennsylvania).

151. Tissue Culture for Hormones in Vivo. W. MCLIMANS (Roswell Park Memorial Institute, Buffalo, New York).

152. Tissue Culture for Hormones in Vivo. E. GOLDWASSER (University of Chicago, Chicago, Illinois).

153. Tissue Culture for Hormones in Vivo. J. C. MITTLER (Veterans Administration Hospital, Detroit, Michigan).

154. Tissue Culture for Hormones in Vivo. G. SATO (University of California, San Diego, California).

155. Invited Symposium: Session III. Oncology, Viruses, and the Cell Cycle. *Chairman:* H. M. JENKINS (University of Minnesota, Austin, Minnesota).

156. Cell Transformation by Viruses. J. L. MELNICK (Baylor College of Medicine, Houston, Texas).

157. The Effects of Viruses on Cellular Biosynthetic Pathways. N. P. SALZMAN (National Institutes of Health, Bethesda, Maryland).

158. Cell Cycle Research, Programmed Macromolecular Synthesis, and the Virus Cancer Problem: Speculations Regarding the Future. G. J. TODARO (National Institutes of Health, Bethesda, Maryland).

Read by Title

159. Liquid Nitrogen Storage of Hormone-secreting Cell Lines. B. H. ATHREYA, A. E. GREENE AND L. L. CORIELL (Institute for Medical Research, Camden, New Jersey).

From naturally occurring tumors, Sato et al. cloned a steroid-secreting mouse adrenal cell (Y_1), a steroid-secreting mouse Leydig Cell (I-10), a steroid-secreting rat Leydig cell (R_2C), an ACTH-secreting mouse pituitary cell (AT_{20}), and a growth hormone-secreting rat pituitary cell (GH_1). In the Cell Bank at the Institute for Medical Research, all of these cell lines continued to secrete their hormones for more than 50 serial passages. All of the cell lines were frozen in Ham's medium containing 10% horse serum and either 10% glycerol or 10% DMSO as the cryoprotective agents and stored in liquid nitrogen. All of these cell lines secrete hormone after storage in liquid nitrogen for 1 year. After thawing and serial subculture, all continue to produce hormone as follows: Y_1 cell line at its 55th passage, I-10 at its 76th passage, R_2C at its 36th passage, AT_{20} at its 25th passage, and the GH_1 at its 56th passage.

160. Effect of Dimethyl Sulfoxide on the Growth, RNA, and Protein Metabolism in Bean Callus Cultures. Y. P. S. Bajaj (Michigan State University, East Lansing, Michigan).

Dimethyl sulfoxide (DMSO) has been reported to change the permeability of plant and animal cells, and has been used as a solvent with agricultural toxicants to check plant diseases. However, no information is available regarding its effect on protein metabolism in plant tissues; such an information would be a prerequisite to any such application. Bean (*Phaseolus vulgaris* L. var. Redkote) stem callus tissues were grown in liquid media containing modified White's minerals supplemented with yeast extract (500 ppm), kinetin (0.1 ppm), and 2,4-D (1 ppm), and the effect of DMSO on their growth, respiration, RNA and protein metabolism was studied. At 0.1 to 0.5% there was no significant effect on growth; however, at 1 to 2% it was reduced by 25 to 50%, and ceased completely at 5% level. At 1% DMSO did not significantly alter the uptake and incorporation of ^{14}C-uracil and ^{14}C-methionine into RNA and protein, although a trend toward inhibition was discernible. At higher concentrations (5 to 10%) DMSO drastically inhibited both ^{14}C-uracil and ^{14}C-methionine uptake and incorporation. Oxygen uptake was inhibited at all concentrations of DMSO, and the inhibition showed a time and concentration dependence. This communication gives an evidence of inhibitory effect of DMSO on respiration, RNA, and protein metabolism, and strongly suggests caution in its use for agricultural purposes.

161. Preservation of Insect Cells in Liquid Nitrogen. B. L. Brown, S. C. Nagle, Jr., J. D. Lehman, and C. D. Rapp (Biological Sciences Laboratories, Department of the Army, Fort Detrick, Frederick, Maryland).

Aedes aegypti cells grown as suspension cultures and *Aedes albopictus* cells grown as monolayer cultures have been frozen and stored in liquid nitrogen with very little loss of viability. Cultures frozen in medium containing 8% dimethyl sulfoxide (DMSO) yielded 93% viable *A. aegypti* cells after storage in liquid nitrogen for 1 month. *A. albopictus* cells preserved similarly yielded a full cell sheet upon thawing within 4 to 6 days and were successfully carried through three transfers with no apparent loss of viability.

162. Preliminary Observations on in Vitro Cancer Chemosensitivity Testing Using Radioactive Tracers.[1] J. E. Byfield, P. E. Byfield, and L. R. Bennett (Department of Radiology, UCLA School of Medicine, Los Angeles, California 90024).

The results of 18 months experience with our previously described (Cancer Res., *28:* 2228, 1968) in vitro cancer cell chemosensitivity assay will be described. A series of biopsies from various gynecologic malignancies of varying histological grades, and including both epidermoid and adenocarcinomas, plus ascitic forms of uterine and ovarian tumors, have been investigated. All tumors evaluated thus far have shown sufficient short term (2-hr) uptake of exogenous labeled nucleoside to allow quantititative comparison of the currently useful anticancer drugs. It will be shown that direct application of the data obtained from isotope uptake inhibition to patient care is not as yet warranted, since the mode of action of individual drugs quantitatively overrides intrinsic variations in tumor chemosensitivity. However, additional experiments relating inhibition of precursor uptake to cytotoxicity in a standard cell line (mouse L1210 leukemia) will be described. The latter experiments, performed in a manner analogous to the biopsy series but including subsequent quantitative studies of actual cytotoxicity, suggest that mathematical correction procedures coupled to acute assays of in vitro cancer chemosensitivity may ultimately yield a quantitatively accurate assay.

163. Observations on the Growth and Degeneration of *Culex tarsalis* Embryonic Cells in in Vitro Cultures.[1] J. Chao and G. H. Ball (U.C.L.A. California).

The best results in attempting to isolate a cell line from *Culex tarsalis* were obtained in cultures of embryonic cells growing in Grace's medium containing 10% FBS and 2% *Samia cynthia* pupal hemolymph. The pattern of growth is different from other mosquito cell lines. Eggs about 36 hr old were surface sterilized with 75% ethanol, followed by washing and homogenation in Rinaldini solution. The debris clot was removed and the cells were spun down, washed, and transferred to flasks for observation under phase contrast on an inverted microscope. Cells 5 to 14 μ in size, some in clusters, became attached within 24 hr. Cell division, contraction, and outgrowth from the clusters soon followed. In about 2 weeks the bottom surface of the flask was criss-crossed by

[1] Supported by funds from the Damon Runyon and James Picker Foundations.

[1] Supported by National Institutes of Health Grant AI-00087.

strands of linked cells, some in single rows, others in bundles. Many were pulsating at about 180 beats per min interpolated with rest periods; others were beating in more complex rhythms. At the same time, patches of cells appeared; many were also contractile. These two tissue-like cell groups increased in size and number for another week. Later, a third type composed of singly attached spindle cells appeared. When trypsinized and subcultured, the cells underwent the same pattern of growth but in decreasing number. Finally, degeneration of the culture occurred with most cell strands and patches detached, the center of the group of the singly attached spindle cells coalescing into multinucleated giant cells reaching sizes of 400 to 500 μ. Two-thirds of the culture medium was usually replenished once a week but the used medium was still adequate to grow Grace's *Aedes aegypti* cells.

164. Production of Infectious Arboviruses and Their Hemagglutinins in BHK-21 Cells. W. A. Chappell, R. F. Toole, and D. R. Sasso (NCDC, Atlanta, Georgia).

A clone of baby hamster kidney cells, BHK-21/13S, was examined to determine its ability to produce a variety of hemagglutinating antigens and infectious virus for the following arboviruses: California encephalitis, Trivittatus, Keystone, La Crosse, Maguari, Oropouche, Wyeomyia, Tensaw, Buttonwillow, Turlock, Shark River, Pahayokee, and St. Louis encephalitis. Most of these viruses were selected because of the difficulty encountered with other methods of preparing hemagglutinating antigens. Suspension cultures or monolayers of BHK-21/13S cells were inoculated with virus and then fed with medium containing 0.4% bovine plasma albumin, instead of serum, and incubated at 35°C. Samples of culture fluids were collected at various intervals and titrated in BHK-21 cells by plaque assay for infectious virus and by microtiter technique for hemagglutinin using goose erythrocytes. Virus multiplication was demonstrated for all viruses studied with peak titers ranging from $10^{5.6}$ to $10^{8.9}$ plaque-forming units per ml. Hemagglutinating antigens were produced for some of the viruses; however, most of these preparations had low HA titers. In some cases HA titers were enhanced by treating cell culture products with Tween 80 and ether. This treatment also eliminated infectious virus from antigen preparations thus making them safer. The most satisfactory hemagglutinin produced was with La Crosse virus which had a titer of 1:128. Suspension cultures of BHK-21/13S cells were therefore shown to be satisfactory for producing large volumes of infectious virus and hemagglutinating antigens of several groups of arboviruses.

165. Inhibitor-like Activity in Nondividing WI-38 Cells. E. Garcia-Giralt, L. Berumen, and A. Macieira-Coelho (Institut de Cancerologie et d'Immunogénétique, Hôpital Paul-Brousse, 94-Villejuif, France).

Human WI-38 cell cultures can be released from growth inhibition under conditions of crowding as has been already demonstrated for other cell systems. In WI-38 cultures, however, a high number of cells respond to the first medium change, the cell population increases although some cells eventually detach, the capacity to be stimulated is progressively exhausted after repeated renewals of medium, and only part of the population responds to further stimulation. The exhaustion of the division potential can be delayed if the cell layer is washed before addition of fresh medium. Supernatants of cultures that no longer respond to medium renewals are inhibitory of the growth of actively growing cultures. The findings suggest that the exhaustion of the division potential after repeated stimulations could be due to the progressive accumulation of an inhibitor. The removal of an inhibitor in perfusion systems in which nutrient medium is frequently renewed might explain the observed uninterrupted cell growth.

166. Response of Tissue Cells to Improved Preparations of Human α_1-Protein Growth Factor. R. Holmes, J. Helms, and G. Mercer. (Alfred I. duPont Institute, Wilmington, Delaware 19899).

It has been suggested that the α_1-protein growth factor alters the permeability characteristics of the cell (J. Cell Biol., *32:* 296, 1967). In the presence of small amounts of this material monolayer cultures of many established cells have been grown in the absence of serum without an adaptive time lag. This time lag often of 120-day duration is eliminated for HeLa cells without the use of α_1-protein if the calcium pantothenate content of the medium is increased from 0.01 mg to 1.0 mg per liter. This is not true for every transformed cell line. For example, Chang's conjunctiva will grow immediately only if in addition to increasing the pantothenate to 1.0 mg per liter a subliminal α-protein is added. The production of α_1-protein by column chromatography is greatly improved by pretreatment of the microbeads with Versene at pH 8.0 and the inclusion of 0.01% Versene in all buffer solutions. Dilute solutions of α_1-protein rapidly lose activity on dialysis and as a consequence short dialysis periods have been recommended. No loss of activity occurs if all dialysis fluids have 0.02% of either sodium thioglycollate or dithioglycol added to them. Both of these substances are demonstrably nontoxic to cells in concentrations as high as 0.1%. There is no evidence for loss of a dialyzable prosthetic group but inactivation is the result of oxidation catalyzed by polyvalent metals such as copper and iron.

167. Importance of Cell Dispersion with Collagenase in the Preparation of Subcultures. E. Y. Lasfargues, W. C. Coutinho, and J. C. Lasfargues (Institute for Medical Research, Camden, New Jersey 08103).

The experiments here described demonstrate that a systematic dispersion of mammary cell cultures with collagenase can maintain the development of

the specific epithelium usually lost in subcultures made by the classical methods of trypsinization. A commercial bacterial collagenase dissolved in Eagles' MEM at the concentration of 132 μ per ml was used; at 37°C, the pooled mammary glands from an adult mouse were completely dissociated in 30 min. When the primary cultures obtained from such a suspension were serially subcultured with trypsin as the dispersion agent, the life span of the cells was limited to about 3 months. Comparatively, collagenase dispersion ensured continuity of cultivation and maintenance of the specific epithelium. Reverting from collagenase to trypsin dispersion at any point of the subculture series resulted in the loss of the epithelium and in the proliferation of the fibroblasts only. Similar observations made with mammary gland cultures from rats and hamsters as well as with human embryonic skin underline the advantages of a more generalized use of collagenase in the practice of subcultures.

168. Influence of Calf Serum on Osteoclasts of Rat Bone Rudiments.[1] M. LISKOVA AND P. JEAN (Département d'Anatomie, Université de Montréal, Montréal, Canada).

Many factors are known to be responsible for the appearance of osteoclasts in bone culture, namely parathyroid extract, vitamins A and D, heparin, and higher oxygen concentrations. Seventy long bone rudiments from 12 19-day rat embryos of the same litter were divided into four groups and cultured during 48 hr. In the control group, bones were cultured in medium M199 Hanks base without serum. In three experimental groups, calf serum at a concentration of 10, 30, or 50% was added to the medium. Osteoclasts were counted on every 10th section throughout each bone. Large osteoclasts showing more than six nuclei on a single section were constantly seen in bones cultured on serum-supplemented media. Although the total number of osteoclasts per bone was not significantly changed, the percentage of large osteoclasts had increased from less than 3% in bones cultivated without serum to an average of 20% in bones of the experimental groups. Osteoclasts seem to form by fusion of mononuclear elements. It is possible that serum added to the culture media contains a factor affecting the properties of cell membranes and favouring the formation of large osteoclasts.

169. Effect of Morphine Sulfate on a Cell Line Derived from Human Glioblastoma Multiforme. L. LISS AND E. H. SUBKO (Laboratory of Neuropathology, Department of Pathology and Psychiatry, The Ohio State University, College of Medicine, Columbus, Ohio).

Several investigators who studied the effect of morphine sulfate on cells in culture concluded that they have observed "addiction" and "withdrawal" effects. Following our acute experiment on the effect of various concentrations of morphine sulfate on cerebellar cultures, we employed our "glioblastoma cell line" as a model. This line is almost 5 years old and has not changed morphologically since the explantation. The cultures under investigation were photographed, filmed, and stained. Morphine sulfate was used in concentrations ranging from 5 mg% to 100 mg%. The drug was added to some of the bottles gradually, employing time tables with target concentrations of morphine between 20 mg% and 100 mg%, while to others the desired concentration was added at once (hit). The experiments were carried for an average period of 3 to 4 months. The results at the present time indicate that: (a) hit of 20 mg% produced morphological alterations (granules, vacuoles, nuclear abnormalities); (b) gradual increase produced the same morphological alterations in concentrations of 25 mg%, the difference in the time exposure being the determining factor; (c) concentrations up to 50 mg% gradually added survived for a total of 3 months. The available results do not indicate development of tolerance to morphine sulfate. Cellular alteration and damage are the function of three factors: (a) concentration, (b) time, and (c) manipulation of the culture. "Conditioning" of culture was apparently of little or no effect. Tolerance to morphine sulfate even if developing was minimal.

170. High and Low Line Cells: Cartesian Diver Studies. J. D. LUTTON[1] AND M. J. KOPAC. (Graduate School of Arts and Science, New York, University, New York).

Two cell types grown in tissue culture, designated high line (NCTC 2472) and low line (NCTC 2555), were studied by Cartesian diver manometric techniques. Micro glass-stoppered divers were used and all loading procedures were conducted under water at 37°C. Cells were maintained in medium NCTC 109 plus 10% fetal calf serum. In divers sealed with 0.18 M $NaHCO_3$ and a gas phase of 5% CO_2 plus 95% air, oxygen consumption was found to remain fairly constant over a 4-hr period. Sodium lauroyl sarcosine, at 0.0018 M, reduces oxygen consumption in the high line cells from 1.09×10^{-5} to 0.38×10^{-5} μl of O_2 per cell per hour, a 65% inhibition. At the same concentration, oxygen consumption in low line cells is reduced from 1.15×10^{-5} to 1.03×10^{-5} μl of O_2 per cell per hour, a 10% inhibition. Both cell lines are almost equally sensitive to 3-nitropropionic acid at 0.0001 M. Anaerobic glycolysis was determined as microliters of CO_2 released from bicarbonate in the presence of 5% CO_2 plus 95% N_2. Anaerobic glycolysis in high line cells is 0.48×10^{-5} μl of CO_2 per cell per hour compared with low line cells which is only 0.17×10^{-5} μl of CO_2 per cell per hour. Anaerobic glycolysis in high line cells is significantly inhibited by iodoacetic acid at 0.001 M (75% inhibition). Mainly, because of the action of sodium lauroyl sarcosine, the high line cells appear to be more

[1] Supported by a grant from the Medical Research Council of Canada.

[1] Damon Runyon Cancer Research Trainee.

dependent on glucose as an energy source than do the low line cells. Both cell lines have almost equally functional succinate systems. The most outstanding difference between the high and low line cells is in the anaerobic glycolytic rates.

171. Lymphocytotherapy of Malignancy. G. E. Moore, R. E. Gerner (N. Y. S. Department of Health, Roswell Park Memorial Institute, 666 Elm St., Buffalo, New York).

The immediate object of lymphocytotherapy is to infuse cancer patients with large amounts of allogeneic and autochthonous cultured human lymphocytes. Thirteen patients have been studied and one, with recurrent malignant melanoma, was infused with over 500 g of lymphocytes. Dramatic regression of the peripheral areas of the tumor growth occurred in this patient but there was no significant change of solid tumor nodules. The residual tumor masses were excised and weekly infusions of cultured lymphocytes continued. Recurrence of tumor growth was noted 3 months after operation. No severe reactions took place and there was no evidence of induced leukemia. Practical and theoretical aspects of lymphocytotherapy include determination of normalcy of the cultured cells, isoantigenicity patterns, the E-B virus, and the danger of graft-host reactions. These preliminary studies of lymphocytotherapy have demonstrated the feasibility of further clinical trials.

172. Characteristics of Primary Tumors Induced by Carcinogenic Polycyclic Hydrocarbons in Hamsters. R. Nelson, P. J. Donovan, and J. A. DiPaolo. (Biology Branch, National Cancer Institute, Bethesda, Maryland 20014).

Recently we have demonstrated that it is possible to obtain quantitative transformation in vitro and proved that cultures derived from the transformed colonies produce tumors when inoculated into hamsters. The purpose of the present study is to determine the degree of similarity of in vitro and in vivo systems of carcinogenesis. Six primary subcutaneous tumors induced with a single injection of benzo(a)pyrene or 7,12-dimethylbenz(a)-anthracene were analyzed for histopathology, growth in vitro, transplantability, and karyology. All tumors were fibrosarcomas with varying degrees of differentiation. Tumor cultures were characterized by rapid growth and acid medium followed by rapid degeneration and survival of a few cells that produced a population that grew indefinitely in vitro. The initial populations were near diploid in mode with numerous acentric pieces and tetrads and dicentric, ring, and marker chromosomes but without any common karyotypic change. Subsequently, there was increased heteroploidy. Cells lacked orientation and occasionally piled up. Cloning without a feeder resulted in diverse colonies, some of which were randomly oriented with crisscrossing of filaments. Tumors derived from these cultures were transplantable into hamsters. These tumors were more anaplastic than primary tumors. Results of these studies imply that in vitro investigations of transformation may provide information relevant to in vivo transformation.

173. The Effect of Tobacco Smoke Components on Plant Tissue Cultures. P. S. Sabharwal and P. R. Bhalla (Botany Department, University of Kentucky, Lexington, Kentucky).

Studies were conducted to determine the effect of tobacco smoke components on the callus tissues of *Nicotiana tabacum.* The callus was obtained from the pith explants of stem. Modified Murashige's medium containing IAA (2 ppm), kinetin (2 ppm), and 2% sucrose served as the control. Different concentrations (ranging from 10^{-4} M to 10^{-10} M) of the following smoke components were utilized: acrolein, benzo(a)pyrene, benzo(e)pyrene, phenol, and pyrene. About 350 to 500 mg of the tobacco pith callus were used as the inoculum. The cultures were observed for 5 weeks for effect on growth caused by the presence of tobacco smoke components in the medium. Benzo(a)pyrene (10^{-7} M, 10^{-8} M) caused extensive callusing of the tissue. Other tobacco smoke components have proved ineffective on this system. These observations clearly demonstrated that very low concentrations of benzo(a)pyrene, a tobacco smoke carcinogen, can induce cell division in plant tissues. Further it is evident from this investigation that plant tissue cultures can be used for a rapid and sensitive bioassay of benzo(a)pyrene.

174. Genetic Suppression of 8-Azahypoxanthine (8-AH) Sensitivity in Human Cells in Vitro. M. W. Steele (Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania).

Human fibroblasts can resist both HAT and 8-AH while HeLa cells can resist HAT but not 8-AH (Steele and Vaughan, 1969). HeLa cells have 3 times more hypoxanthine phosphoribosyl transferase activity (HGPRT) than fibroblasts, but that cannot explain this phenomenon since human quasi-diploid RPMI 2650 cells have similar HGPRT activity as HeLa cells but resist 8-AH. These observations suggest that HeLa cells lack an active genetic locus, or loci, which is active in human diploid cells, is independent of the HGPRT locus, and which positively suppresses sensitivity to 8-AH. The following supports this: D98/AH-2 cells ($HGPRT^-$, heat-labile G6PD, 8-AH-resistant, HAT-sensitive) were mixed with HeLa S3 cells ($HGPRT^+$, heat-stable G6PD, 8-AH sensitive, HAT-resistant). The loci for HGPRT and G6PD are both X-linked. After 2 weeks in MEM, the mixture was placed in HAT for 4 weeks, then in MEM + 100 μg per ml of 8-AH for 4 weeks, then in HAT + 100 μg per ml of 8-AH. In the latter media, about 75% of the culture died but numerous healthy clones survived. Confluent cultures of the latter in HAT + 8-AH had 2X normal HGPRT activity and intermediate G6PD heat stability. Hence, X chromosomes from both HeLa S3 and D98/AH-2 cells were present. Cloning efficiency of these cells in HAT + 8-AH was 13%. These data suggest that: HeLa S3, D98/AH-2, and

hybrid cells were all living in HAT + 8-AH; the latter two resist HAT due to HGPRT supplied (as the enzyme or as information) by HeLa S3 cells via cell contact and/or hybridization; the HeLa S3 cells resist 8-AH because of a substance, or substances, generated in D98/AH-2 cells which is transferred (as already synthesized or as information) to HeLa S3 cells by cell contact and/or hybridization.

175. Failure of Tricine-buffered Medium to Support Growth of Human Diploid Fibroblasts. S. WOOD AND L. PINSKY (Lady Davis Institute for Medical Research, Jewish General Hospital, and Human Genetics Sector, McGill University, Montreal Canada).

Nonvolatile buffers are advantageous for experimental manipulation of open culture systems in order to avoid pH fluctuation. Several amine buffers with suitable pK values have been successfully used with various heteroploid cell lines. Consequently we have tested the ability of Tricine-buffered medium to support propagation of human diploid cells. The incorporation of ^{3}H-thymidine and ^{14}C-reconstituted protein hydrolysate was used to estimate the rate of cell division and the population size per culture. A novel procedure for preparing an acid-insoluble cell fraction for counting was used, wherein all manipulations were carried out directly on the monolayer adhering to a plastic culture dish. Tricine-buffered medium did not support cell growth. The effect of Tricine seems to be a reversible inhibition of mitosis since explants cultured in Tricine-buffered medium were noted to resume mitosis after being transferred to bicarbonate-buffered medium.