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REPOSITORY Oak Ridge Operations Records Holding Area
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BOX No. H-138-1 Bldg. 2714-H
FOLDER 40-1669-85 Oct. 1985

SEP 0 8 1986

Ms. Dorothy G. Williams
Contracts Management Office
National Institute of Environmental Health Sciences
Post Office Box 12874
4310 South Miami Boulevard
Research Triangle Park, North Carolina 27709

Dear Ms. Williams:

DOE ASSISTANCE TO NIEHS, "DEVELOPMENT OF IMAGE ANALYSIS METHODOLOGY FOR COMPARATIVE ANALYSIS OF PROTEINS FROM TWO-DIMENSIONAL GEL ELECTROPHORESIS" (INTERAGENCY AGREEMENT DOE NO. 40-1669-85)

Enclosed for your consideration are copies of an Interagency Agreement and proposal. If the Interagency Agreement meets your approval, please return a copy signed on behalf of your organization to this office. The proposed work continues this project for one year with funding of $246,519.

On future funding documents, please reference the Interagency Agreement number (40-1669-85) and identify specific sections (or subtasks) of the proposal that are being authorized for initiations. If you elect to provide a separate task statement, you must identify the applicable proposal section and provide an assessment of how your task statement differs from the proposed work. If the information discussed above is not provided, we will not be able to accept your funding document. If, in the future, there is a need to change the (1) Statement of Work, (2) performance period and/or (3) funding level, please address all correspondence or funding documents to me. Upon our acceptance we will authorize ORNL to proceed with the research project. Monthly billings will be accomplished by the Simplified Intragovernmental Billing and Collection (SIBAC) system to reimburse the DOE appropriation for actual cost.

1051991

Technical question concerning the proposal should be directed to R. J. M. Fry, telephone (615) 574-1252, of ORNL. Administrative questions should be addressed to Robin Spradlen, DOE Work for Others Coordinator, telephone (615) 576-0646.

Sincerely,

Original Signed by
William R. Bibb

William R. Bibb, Director
Research and Waste Management Division

ER-122:Spradlen

Enclosures:
1. Interagency Agreement (2)
2. Proposal (7)

cc w/encls:
D. H. Moneyhun, DOE-OSTI (ORO-UT)

cc wo/encls:
H. Postma, ORNL
R. J. M. Fry, ORNL
J. E. Carr, ORNL

| CONCURRENCES | |
|---|---|
| RTG. SYMBOL | ER-122 Spradle |
| INITIALS/SIG. | RQS |
| DATE | 9/2/86 |
| RTG. SYMBOL | ER-121 Engre |
| INITIALS/SIG. | [illegible] |
| DATE | 9/4/86 |
| RTG. SYMBOL | ER-122 Radcli |
| INITIALS/SIG. | [illegible] |
| DATE | 9/5 |
| RTG. SYMBOL | ER-12 Bibb |
| INITIALS/SIG. | WRB |
| DATE | 9-8-86 |
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**OAK RIDGE NATIONAL LABORATORY**
OPERATED BY MARTIN MARIETTA ENERGY SYSTEMS, INC.

POST OFFICE BOX X
OAK RIDGE, TENNESSEE 37831

August 28, 1986

Mr. Joseph A. Lenhard, Assistant Manager
Energy Research and Development
Department of Energy, Oak Ridge Operations
Post Office Box E
Oak Ridge, Tennessee 37831

Dear Mr. Lenhard:

Revised Proposal to the National Institute of Environmental Health Sciences (NIEHS), "Development of Image Analysis Methodology for Comparative Analysis of Proteins from Two-Dimensional Gel Electrophoresis," (Interagency Agreement No. 40-1669-85)

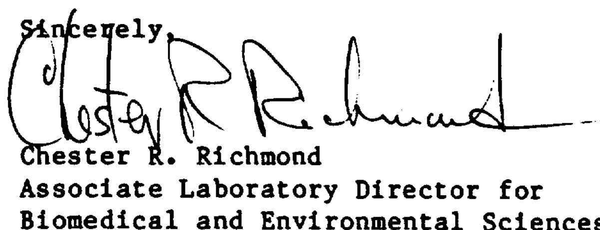
Enclosed for your review and approval is a revised research proposal for submission to the National Institute of Environmental Health Sciences (NIEHS). The proposed research is based on discussions between Dr. James Selkirk of NIEHS and personnel from the Biology Division at Oak Ridge National Laboratory (ORNL). The proposal requests a performance period of one year beginning October 1, 1986. The total estimated cost for the effort is $246,518.

The objective of this study is to monitor early changes in multiple cellular enzymes and proteins after carcinogen treatment with ability to store, rapidly retrieve and analytically contrast the effects of various chemical treatments. The proposal is directly related to DOE Activity HA 02 02 01 0, FTP/A No. 004452, "Carcinogen Activity in Human Cells." Existing space and resources are available to conduct this work without interfering with Department of Energy efforts. This research is described in the approved ORNL Institutional Plan on page 67.

After your approval, please forward the original plus six (6) copies to Ms. Dorothy G. Williams, National Institute of Environmental Health Sciences, Contracts Management Office, Post Office Box 12874, 4310 South Miami Boulevard, Research Triangle Park, N.C. 27709.

If you have any questions concerning this proposal, please contact Dr. R. J. M. Fry, extension 4-1252.

Sincerely,



Chester R. Richmond
Associate Laboratory Director for
Biomedical and Environmental Sciences

Enclosure

cc: J. E. Carr, R. J. M. Fry, R. A. Griesemer, D. S. Leggett, H. Postma - RC, C. S. Travaglini

1051993

1051994

1051995

William Bible

9-8-86

1051996

# NATIONAL TOXICOLOGY PROGRAM

Title: Development of Image Processing Methodology for Comparative Analysis of Proteins from Two-Dimensional Gel Electrophoresis: Studies to Monitor Early Changes in Multiple Cellular Enzymes and Proteins after Carcinogen Treatment, with the Ability to Store, Rapidly Retrieve and Analytically Contrast the Effects of Various Chemical Treatments.

Period of Award: September 30, 1986 - September 29, 1987

Funding Mechanism: Interagency Agreement

Objective:

To determine the critical metabolic parameters that differentiate between chemical carcinogen susceptible and resistant cells.

Background:

Chemical carcinogens and mutagens are metabolized by a complex of enzymes comprised of the monooxygenases as the activation fraction, and the epoxide hydrolases and cytoplasmic transferases as the detoxification fraction. The primary function of the metabolic processing of xenobiotics is to render them biochemically inert and water soluble for more facile excretion from the organism. This enzymatic array has evolved as the major mode of detoxifying chemicals, and while the liver is the main site of this activity, the monooxygenase system appears to be ubiquitous in mammalian tissues. Since many enviromentally prevalent toxic substances are chemically stable it is necessary for the tissues to deactivate them by derivatization into innocuous forms (Coon et al., 1980). However, cellular processing for parent carcinogens and mutagens toward detoxification form the highly reactive intermediates that are now known to be the carcinogenic and mutagenic forms of the molecule (Harris and Cerrutti, 1982). The data base on carcinogen metabolism has grown substantially over the last decade, and metabolite profiles for various chemicals from tissues and cells in many species have been elucidated. From this vast compendium of data two generalizations have been derived. It appears that all active carcinogens are electrophiles (Miller and Miller, 1977) and all species form the same family of metabolites for any given chemical carcinogen (Selkirk et al., 1982). Unfortunately, for a given carcinogen, we are then faced with the reality that our current theoretical concepts and experimental metabolic data base cannot explain the reasons for the wide range of susceptibility to malignant transformation by a given chemical among test species. Since all short-term and long-term carcinogen and mutagen assays rely on the same type of metabolic activation system (Douglas, 1984), it is necessary to probe further into the metabolic reasons for the relative differences in susceptibility for a wide spectrum of chemical carcinogens. The valid extrapolation of animal carcinogenicity data to human risk requires the most complete understanding of the respective differences and similarities in the processing rate or specific modulation of the critical enzymes for activation and detoxification.

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Rationale:

The burgeoning chemical carcinogen data base clearly shows all metabolic products are identical for a given chemical in all eukaryotes and have markedly similar patterns of macromolecular binding. With the exception of a single generalization that all chemical carcinogens form reactive electrophiles, there is no unifying concept to explain the specific carcinogenic mechanism for chemicals of widely divergent structure. The large number of metabolic studies over the last decade exhibit a marked phylogenetic conservation for xenobiotic metabolism with similar activation and detoxification pathways. Yet there is a wide spectrum of relative susceptibility and resistance to malignant transformation between species, tissues and cells for carcinogens and mutagens (Langenbach et al., 1983). Furthermore, there is great variation in susceptibility between species for a given carcinogen. Tumorigenesis assays best exemplify the concept of variable susceptibility. Specialized assay systems have evolved for often unknown biochemical reasons, which are uniquely sensitive to a given chemical class or mode of treatment. The mouse skin model for initiation/ promotion and the rat mammary tumor system are excellent for polycyclic carcinogens, while other species are not as sensitive to these chemicals. Another example of unique sensitivity is aflatoxin B1 where rat, ferret and trout develop liver tumors at microgram doses while the mouse is highly resistant to hepatoma formation by this chemical.

This metabolic enigma of an apparent identity in overall metabolism with a concommitant susceptibility variance for malignant transformation in different species has eluded explanation. It becomes critical to attempt to understand the biochemical and genetic mechanisms for this paradoxical situation where a given carcinogen will produce the putative reactive intermediate in two test species and will exhibit a clear dose response in one species and be completely refractory in the other. It is becoming increasingly important to understand if species variance to chemical carcinogenesis is a function of the genetic regulation of the vast array of enzymes induced during activation and detoxification of the carcinogen.

The goal of this project is to determine which portions of the metabolic pathways are more critical in forming, maintaining, and directing the ultimate carcinogenic or mutagenic species of the molecule toward its final biological fate. In the past it was only possible and practical to monitor a few enzymes in the metabolic scheme. Standard assays exist for only a small number of the components and are sufficiently labor intensive to prohibit large experiments that would include many time points and various chemical treatments. However, an analytical system that could simultaneously assay all the critical cellular proteins would markedly enhance our understanding of the kinetics of each component in the enzymatic pathway by determining which proteins are altered by chemical treatment, and how susceptible and resistant cells differ in carcinogen processing.

1051968

Approach:

The proposed research will establish an analytical system that is capable of qualitatively and quantitatively measuring cellular proteins. Using

two-dimensinal gel electrophoresis and computer image analysis technology it will be possible to accurately monitor pertubations in the array of cellular proteins during chemical carcinogen treatment. Since both susceptible and resistant cells to malignant transformation have been shown to produce the identical array of metabolites for any given carcinogen, the enzymatic processing of the chemical may be altered in such a way as to shunt the metabolism of the carcinogen toward or away from formation of the reactive intermediate. By monitoring the enzyme systems responsible for activation and detoxification in the cell and their alterations by both carcinogenic and noncarcinogenic isomers, it is expected that the pathways critical for activation and detoxification will be discernible when compared to identical protein profiles from untreated cells.

However, it is first necessary to develop the methodology to its maximum level of reproducibility. Therefore this project will compare the protein profiles of both human and rodent cells to ascertain basic differences in the cytoplasmic and nuclear protein composition in control and chemically treated cells. The plan will be first to prepare master maps of selected rodent and human cellular proteins. These profiles will be digitized, processed in an image analyzer, and stored in the computer for comparison against the protein maps from carcinogen-treated cells. Special image analysis algorithms will determine qualitative and quantitative pertubations in cellular proteins between control and chemically treated cells. The first chemical probes in this research will be the use of carcinogenic and noncarcinogenic isomers of the same polycyclic hydrocarbon molecule. Benzo(a)pyrene and dibenz(a,h)anthracene and their noncarcinogenic congeners benzo(e)pyrene and dibenz(a,c)anthracene are excellent probes since the latter compounds have been predicted by the current bay-region hypothesis to possess significant carcinogenic potential. However, in vivo and in vitro studies have shown benzo(e)pyrene and dibenz(a,c)anthracene to be inactive as initiators.

## Background History and Needs

Chemical carcinogens and mutagens are metabolized by a complex of enzymes comprised of the monooxygenases as the activation fraction, and the epoxide hydrolases and cytoplasmic transferases as the detoxification fraction. The primary function of the metabolic processing of zenobiotics is to render them biochemically inert and water soluble for more facile excretion from the organism. This enzymatic array has evolved as the major mode of detoxifying chemicals, and while the liver is the main site of this activity, the monooxygenase system appears to be ubiquitous in mammalian tissues. Since many environmentally prevalent toxic substances are chemically stable it is necessary for the tissues to deactivate them by derivatization into innocuous forms (Coon et al., 1980). However, cellular processing for parent carcinogens and mutagens toward detoxification form the highly reactive intermediates that are now known to be the carcinogenic and mutagenic forms of the molecule (Harris and Cerrutti, 1982). The database on carcinogen metabolism has grown substantially over the last decade, and metabolite profiles for various

chemicals from tissues and cells in many species have been elucidated. From this vast compendium of data two generalizations have been derived. It appears that all active carcinogens are electrophiles (Miller and Miller, 1977) and all species form the same family of metabolites for any given chemical carcinogen (Selkirk et al., 1982). Unfortunately, for a given carcinogen, we are then faced with the reality that our current theoretical concepts and experimental metabolic database cannot explain the reasons for the wide range of susceptibility to malignant transformation by a given chemical among test species. Since all short-term and long-term carcinogen and mutagen assays rely on the same type of metabolic activation system (Douglas, 1984) it is necessary to probe further into the metabolic reasons for the relative differences in susceptibility for a wide spectrum of chemical carcinogens. The valid extrapolation of animal carcinogenicity data to human risk requires the most complete understanding of the respective differences and similarities in the processing rate or specific modulation of the critical enzymes for activation and detoxification.

## Data, Data Rights, Patents, and Copyrights

All data and materials shall be available to the National Toxicology Program project officer and/or his designee for inspection or use at any time during the IAA. The data and samples shall be stored in the Biology Division of the Oak Ridge National Laboratory through the term of the IAA. The data set includes proteins extracted from cells and tissues and processed in the Biology Division, all relevant data stored in the computer, protocols and standard operating procedures developed by the laboratory, data generated and collected, and reports and manuscripts prepared by the Biology Division on this project.

At the conclusion of the project the data shall be shipped to the NTP unless the project officer and principal investigator agree that it is in the best interest of the project to leave some or all of the data, or copies of the data, in the Biology Division at ORNL. Data refers to records on paper and/or other collection media, but as such not limited to computer disks, tapes, or microfiche.

The agency shall acknowledge the support of the Department of Health and Human Services, National Institutes of Health, National Institute of Environmental Health Sciences, whenever publicizing the work under this agreement in any media. Support shall be acknowledged as follows:

"This work was conducted for the National Toxicology Program under the National Institute of Environment Health Sciences, Interagency Agreement No. YO1-ES- ."

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## Replication, Dissemination, or Use of the Results

These results should be presented for use of the general chemical carcinogenesis community to help understand the mechanism of action of chemical carcinogen processing in cells susceptible to malignant transformation. The data should be published as collaborative efforts between the principal investigator and the project officer. No information may be released however without prior written authorization by the NTP project officer.

## Post-Award Administration and Monitoring

MILESTONES

1. Contract Award (Day 0).

2. Gel equipment will be utlized and the system fine tuned for reproducibly electrophoresing 20 two-dimensional gels simultaneously. Algorithms will be prepared for developing master control gel images, and the ability to search multiple gels for specific proteins will be obtained.

3. The principal investigator will submit a semiannual report.

4. Final report reviewing the results of all studies (by the end of the interagency agreement term).

## Statement of Work

The National Toxicology Program utilizes various short-term and chronic assays for toxicity, carcinogenesis, and genotoxic effects. For the vast majority of environmentally prevalent chemicals, some form of metabolic activation is required by the cell's microsomal, monooxygenase complex to form reactive intermediates for manifestation of the toxic principle. This research program is designed to broaden the empirical approach to assessing the role of metabolism of chemicals in malignant transformation susceptible and resistant cells. The overall plan is to utilize both cell types as probes to understand why certain species, tissues, and

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cells are predisposed toward malignant transformation by chemicals and compare the metabolic competence, and changes in the cellular metabolic machinery during activation and detoxification of the carcinogens.

Cell Culture

Studies are to be initiated with the C3H mouse embryonic (10T½) fibroblast line, which is highly reliable for malignant transformation studies by chemicals. The cultures are maintained in Eagle's basal medium with 10% heat inactivated fetal calf serum, glutamine, 2 mM and penicillin (110 units/ml) and streptomycin (110 mcg/ml). Cells are passaged every 10 days and plated at 0.5 x $10^{-5}$ cells/60-mm dish. Media changes are made at 5-day intervals and the cells are maintained at 37°C and 7% $CO_2$ in air. Cell cultures will be used between the fifth and eleventh passage for optimum transformation capability. Human foreskins are obtained courtesy of the Oak Ridge Methodist Hospital. The tissue is cut into small pieces and plated in Dulbecco's medium with high glucose and antibiotics as above. Fibroblasts grow out from the explants and are passaged with mild trypsinization.

Transformation Assays

Transformation assays will be performed according to Reznikoff et al. (1973) in the following manner. Thirty 60-mm dishes are seeded at 1000 cells each from freshly confluent cultures in 5-ml medium. Thirty media controls and 30 DMSO (0.5%) controls are included. Exposure times are 24 hrs, 48 hrs, and 6 days. Medium is changed twice weekly until the dishes reach confluency and then once weekly. After 6 weeks the medium is removed and the cells washed with phosphate buffered saline, fixed with methanol, and stained with Giemsa, and scored for transformed foci. Numbers will be reported as transformed foci (types I, II, or III) per dish. Cytotoxicity will be performed in the following manner: In one assay cells are seeded at 200 cells/60-mm dish in separate groups of 10 dishes for carcinogen-treated and DMSO solvent control. Between 7 and 10 days the medium is removed and the dishes stained with Giemsa. Both control and treated colonies are counted for plating efficiency determination in the following manner:

Percent plating efficiency = number of colonies/dish X 100/200 (seeded cells).

Percent plating efficiency = PE (treated) X 100/PE (control).

For the second cytotoxicity assay five dishes (1000 cells/60-mm dish) each for chemical treatment and DMSO control are used. After the designated incubation period the cells are lightly trypsinized (0.05%/5 min) and counted in either a hemacytometer or Coulter counter. Cytotoxicity is expressed as follows:

Percent toxicity = the number of cells treated X 100/control.

1052002

## Cytoplasmic and Nuclear Protein Preparation

Radiolabeled cytoplasmic and nuclear proteins are isolated from cells grown to confluence on 100-mm tissue culture plates according to a modification of the method described by Berezney and Coffey (1977). Buffers are used at $4^{o}C$ and contain 0.5 mM phenylmethyl sulfonyl fluoride (PMSF) to inhibit protease activity. The cells are lysed using a Dounce homogenizer in 10 mM Tris pH 7.4. The crude nuclear preparation is then pelleted at 100 xg for 10 min, resuspended in Tris, homogenized, and pelleted once more. The supernatant cytoplasmic fractions are combined and dialyzed twice against 100 volumes of triple-distilled water containing 0.5 mM PMSF at $4^{o}C$ and finally frozen and lyophilized. Proteins are quantitated according to the fluorescamine assay (Weigele et al., 1972) before electrophoresis. The electrophoretic assay can be simplified by subfractionation of the cytoplasmic components by differential centrifugation (15,000 g = mitochondrial, 100,000 g = microsomal, 100,000g supernatant = soluble enzymes). Each cytoplasmic protein fraction is dialyzed, lyophilized, and quantitated before being analyzed by two-dimensional gel electrophoresis. Crude nuclear pellets derived from the cytoplasmic protein isolation procedure above are then suspended 2.2 mM sucrose, 50 mM Tris, pH 7.4, 5 mM $MgCl_2$, and centrifuged at 40,000 g for 90 min in a Beckman 50Ti rotor to shear off cytoplasmic protein adhering to the nuclear membrane. The nuclear pellet is suspended, washed, and centrifuged at 800 x g two times in 0.25 M sucrose, 50 mM Tris, pH 7.4, and 5 mM $MgCl_2$. Total nuclear proteins can then be solubilized in 6 M guanidine hydrochloride, 10 mM EDTA, pH 7.0, and sonicated in 30 sec bursts at ~20 watts with a Branson sonifier. The residue is then dialyzed, lyophilized, quantitated, and prepared for isoelectric focusing gels.

## Two-Dimensional Gel Electrophoresis of Cytoplasmic and Nuclear Protein Fractions

### First Dimension: Isoelectric Focusing

Carbon-14 amino-acid labeled cytoplasmic and nuclear proteins from cells grown in culture are analyzed using a modification of the O'Farrell high resolution two-dimensional electrophoresis technique (O'Farrell, 1975). Routinely, 100 g cellular protein fractions (prepared as described) are dissolved in 10 l lysis buffer (9.5 M urea, 2.0% NP-40, 2% Ampholytes pH 3.5-10 (LKB), 5.0% beta-mercaptoethanol) and loaded on each isoelectric focusing tube gel (gel composition: 9.17 M urea, 2% Ampholytes pH 3.5-10, 4% acrylamide, 6% bis-acrylamide, 0.2% NP-40, 0.02% ammonium persulfate, and 0.0013% TEMED; gel size: 1.5 mm i.d. x 130 mm). The cathode solution is 30 mM NaOH and the anode solution is 10 mM $H_3PO_4$. The samples are loaded under the cathode solution and isoelectrically focused for a total of 690 vhr/cm. The initial 75% of the volt-hrs are run at 450 volts and the final 25% of the volt-hrs are run at 2000 volts for band sharpening.

### Second Dimension: SDS Polyacrylamide Slab Gel Electrophoresis

After electrofocusing, the first dimension gels are soaked in equilibration buffer (10% glycerol, 8.6 mM dithiolthreitol, 2% SDS, 0.125 M Tris-HCl, pH 8.6 for 10 min and then loaded directly onto the top of the second dimension (160 X 140 X 1.5 mm) gel. Standards are placed alongside

1052003

the tube gels as a molecular weight reference. The second dimension gel is composed of a 10-20% polyacrylamide gradient, (Anderson et al., 1978). The running buffer is composed of 0.025 M Tris pH 8.3, 0.192 M glycine, and 0.1% SDS. Electrophoresis is carried out to 175 ma-hr/gel slab. Twelve such slab gels are run simultaneously for reproducibility. After electrophoresis, the proteins within the slab gel are then precipitated in 20% Ethanol, 5% acetic acid, and 2.5% sulfosalicylic acid.

The gels are either silver stained or prepared for fluorography (using the fluors supplied in Amplify by Amersham). Kodak SB X-ray film is preflashed to an optical density of 0.15 and sandwiched with the dried fluor-impregnated gel and radioactivity calibration strips (for radioactivity quantitation in protein spots) and exposed at $-80^{o}$C for the appropriate time period, developed and scanned on the drum scanner.

Scanning Densitometry and Two Dimensional Gel Electrophoresis Image Processing

The developed X-ray film containing the 2D gel image and radioactivity calibration strips is digitized at 100 microns resolution on an Optronics Photoscan P-1000 Model 30-D drum scanner. The original scanner data, of approximately 2.4 M bytes are stored on a 457 M byte disk unit under the control of a PDP11/70 computer operating under RSX11M+. Subsequently the images are analyzed using an $I^2S$ Model 75 image processing system (International Imaging Systems, Milpitas, California). The image analysis algorithms include non-linear filters for background radioactivity removal, routines to detect and characterize each protein spot by a small set of parameters that describe shape, size, and spatial location on the gel, and give an intensity estimate for quantitation based on radioactivity incorporation into the protein. This reduced data set, as few as 5 values per spot are possible, defines each protein spot on an image and is stored on disk to form part of an image data base for comparison between various carcinogen treatments. In order that many different film images can be compared within the data base, each image must be registered to reference spots on the master image composite. A rubber-sheet warping algorithm (already in use) is employed by the image processor which aligns and registers the composite and experimental images. It is the reduced data set which is actually used in making comparisons of protein perturbations in the variant cell populations after carcinogen treatment.

For the growing data base, algorithms are being developed to direct the host computer to search and compare images produced in control experiments to those images from cells treated with carcinogen, promoters and other chemicals. A technique that involves generating ratios of one image region with respect to another will be utilized in our application to analyze relative radioactive label intensity among proteins of interest.

Time course studies will be performed over various incubation periods to follow the rate of induction of cellular proteins involved in carcinogen activation and detoxification. When the data assembled in the computer reaches a significant level, we will attempt to develop a kinetic description of induction for these critical enzymes in the cell for carcinogen processing in both resistant and susceptible cells to malignant transformation. Such a model will help define the dynamic differences between the metabolism of

1052004

susceptibility that results in malignant transformation and a mechanism of resistance where the cell has succeeded in repairing the damage. The commonality of reactive intermediates in all species can than be viewed from the vantage point of a more complete knowledge of how cells selectively process carcinogens through the many available metabolic directions.

Personnel Requirements and Level of Effort

The proposed project requires three distinct types of expertise: An individual highly trained in mathematics of image analysis technology with a strong background in computer science (it is anticipated that this requires a doctoral level position); in addition there is need for two technical staff -- one with experience in isoelectric focusing and two-dimensional gel electrophoresis of proteins, and the second individual, a cell culture technologist, with experience in in vitro transformation and mutagenesis, metabolism in vitro and the establishment of routine primary cultures from rodents. In addition, this individual must have some experience with the use of a fluorescence activated cell sorter. It is anticipated the latter two individuals will utilize 100% of their time on this project. The first individual will provide 10% effort on this project.

Travel Requirements

Funds will be needed for quarterly visits (one person) on the project to NIEHS. Each visit will last for not more than two days.

Award of Contracts

It is anticipated one interagency agreement will be awarded.

Deliverable Reporting Requirement

Description report on current level of research concerning development of analytical algorithms and progress in the increased capability of running multiple two-dimensional gels with higher degrees of reproducibility. Quantity to be determined. Date due semiannual. Delivery address will be to Project Officer, Dr. James K. Selkirk, Carcinogenesis and Toxicology Evaluation Branch, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, North Carolina 27709. A final report shall be due on or before the agreement expiration date.

Project Officer and Alternate Project Officer

Project Officer: Dr. James K. Selkirk, Carcinogenesis and Toxicology Evaluation Branch, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, North Carolina 27709.

1052005

Reference Material

References:

Schaefer, E. L. and Selkirk, J. K. Metabolism of benzo(a)pyrene by variant mouse hepatoma cells. Cancer Res. 45: 3487-3492, 1985.

Schaefer, E. L., Au, W. W. and Selkirk,, J. K. Differential induction of sister-chromatid exchanges by benzo(a)pyrene in variant mouse hepatoma cells. Mutation Res. 143: 69-74, 1985.

Coon, M. J., et al. (Eds.), Microsomes, Drug Oxidations, and Chemical Carcinogenesis, Vols. I and II. Academic Press, New York, 1980.

Harris, C. C. and Cerutti, P. A. (Eds.), Mechanisms of Chemical Carcinogenesis. Alan R. Liss, New York, 1982.

Miller, S. A. and Miller, E. C. Ultimate chemical carcinogens as reactive electrophiles. In, Origins of Human Cancer, Vol. B, H. H. Hiatt, J. D. Watson and J. Winsten (Eds.). Cold Spring Harbor Lab., New York, pp. 605-627, 1977.

Douglas, J. F. (Ed.), Carcinogenesis and Mutagenesis Testing. Humana Press, Clifton, New Jersey, 1984.

Mann, R. C., Mansfield, B. K., and Selkirk, J. K. Automated analysis of digital images generated by two-dimensional gel electrophoresis. Proc. Conf. on Pattern Recognition in Practice II. North Holland Pub. Co., in press.

Langenbach, R., Nesnow, S., and Rice, J. M. (Eds.), Organ and Species Specificity in Chemical Carcinogenesis. Plenum Press, New York, 1983.

Reznikoff, C. A., Bertram, J. S., Brankow, D. W., Heidelberger, C. Quantitative and qualitative studies of chemical transformation of cloned C3H mouse embryo cells sensitive to past confluence inhibition of cell division. Cancer Res. 33: 3239-3249, 1973.

Berezney, R. and Coffey, D. S. Nuclear matrix isolation and characterization of a framework structure from rat liver nuclei. J. Cell Biology 73: 616-627, 1977.

Weigele M., DeBernado, S., Terigi, J. and Leingruber, W. A novel reagent for the fluorometric assay of primary amines. J. Amer. Chem. Soc. 94: 5927-5930, 1972.

O'Farrell, P. H. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. 250: 4007-4021, 1975.

1052006

Anderson, N. G. and Anderson, N. L. Analytical techniques for all fractions. XXI. Two dimensional analysis of serum and tissue proteins. Multiple isoelectric focusing. Analytical Biochemistry 85: 331-340, 1978.

Other Pertinent Information

This project requires utilization of mathematical, biochemical, and cellular biological skills in order to develop a biochemical scheme that monitors changes in susceptible and resistant cells as carcinogens or other toxic chemicals are carried through the activation and detoxification process. The project will require close collaboration between the technical and professional individuals. The cell biology, the biochemistry, and the mathematical development of the data can be closely aligned so a mechanism for biochemical toxicology can be assembled when a sufficiently large data base is developed for numerous resistant and susceptible cells in a series of known chemical carcinogens of widely divergent structure.

1052007

PROPOSED BUDGET

| Staff | MY | $ |
|---|---|---|
| Professional | | |
| Dr. R. Mann | 0.1 | $5,824 |
| Technicians | | |
| A. Noghrei-Nikbakht | 1.0 | 29,120 |
| B. Mansfield | 1.0 | 29,120 |
| Total Staff | 2.1 | $64,064 |
| **Other Direct Cost** | | |
| Fringe Benefits @ 22.0% of Labor | | $14,094 |
| Division Administration @ 32.0% of Labor | | 20,500 |
| *Materials/Supplies | | 24,487 |
| Animals | | 10,260 |
| Utilities | | 51,253 |
| Total Other Direct Cost | | $120,594 |
| TOTAL DIRECT COST | | $184,658 |
| **Indirect Cost** | | |
| Laboratory Overhead @ 33.5% of Total Direct Cost | | $61,860 |
| TOTAL PROJECT COST | | $246,518 |

*See detailed budget

1052008

EXPLANATION

STAFF:

Man-year cost is based on 2,080 hours per year. This figure would include any absence time (sick, vacation, etc.).

OTHER DIRECT COST:

Fringe Benefits - Reflects the Company's cost of employee plans (group insurance, savings plan, dental insurance, pension plans) and the legally required benefits (FICA taxes, unemployment compensation, worker's compensation, public liability insurance). These costs are collected and distributed monthly on the basis of labor. The fringe benefit rate is projected to be 22.0% for the duration of the proposed project. In the ORNL Uniform Accounting System, the actual rate will be charged monthly. (22% x $64,064 = $14,094)

Division Administration - Essentially a Division overhead cost. Costs related to Division management are collected here and distributed monthly to the Division Cost Collect Centers on the basis of labor. A rate of 32.0% is projected for the duration of the proposed project. The major cost elements are the salaries and fringe benefits of the following personnel: Division Director, Safety and Radiation Control Officer, Receptionist, Purchasing (2), Technical Secretarial staff (10), Administrative and Budget (4), and Editorial (1). Telecommunications charges are also collected here. (32% x $64,064 = $20,500)

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Materials and Supplies

OTHER DIRECT COSTS - $24,487

| | Quantity | Unit Cost | Amount |
|---|---|---|---|
| **Tissue Culture and Associated Expendibles** | | | |
| 60 mm TC Plates | 8 cases | $127.00/case | $1016 |
| 100 mm TC Plates | 3 cases | 163.00/case | 489 |
| Sterile Pipettes: | | | |
| 25 ml capacity | 54 pkg | 2.50/pkg | 135 |
| 10 ml " | 20 pkg | 10.04/pkg | 201 |
| 5 ml " | 12 pkg | 6.00/pkg | 72 |
| 1 ml " | 4 pkg | 14.00/pkg | 56 |
| Pasteur Pipettes (9") | 2 boxes | 6.76/box | 14 |
| " " (5") | 8 boxes | 2.06/box | 16 |
| Pipette Tips | 4 bags | 10.00/bag | 40 |
| Sterile Centrifuge Tubes (50 ml) | 2 cases | 109.88/case | 220 |
| " " " (15 ml) | 3 cases | 90.00/case | 270 |
| BME Medium | 6 boxes | 10.90/box | 65 |
| DME " | 6 boxs | 10.90/box | 65 |
| Gentamicin | 2 units | 53.90/unit | 108 |
| | TISSUE CULTURE SUBTOTAL | | $2,767 |
| **Radioisotopes** | | | |
| [$^{14}$C]Amino Acids | 7 mCi | $425.00/mCi | $2,975 |
| [$^{3}$H]Benzo(a)Pyrene | 25 mCi | 400.00/25 mCi | 400 |
| | RADIOISOTOPES SUBTOTAL | | $3,375 |
| **Two-Dimensional Gel Electrophoresis and Associated Expendibles** | | | |
| Protein Markers | 2 kits | $35.00/kit | 70 |
| (PMSF) Phenyl Methyl Sulfonyl Fluoride | 25 g | 66.09/25 oz | 66 |
| (HMBA) Hexamethylene-Bis-Acetamide | 50 g | 23.00/50 g | 23 |
| Benzidine | 400 mg | 13.00/100 mg | 52 |
| Tris Base | 2000 g | 16.00/500 g | 64 |
| Glycerol | 3000 ml | 14.00/500 ml | 84 |
| Ethanol | 30 gal | 5.00/gal | 150 |
| 5-Sulfosalicylic Acid | 3000 g | 16.00/500 g | 96 |
| Dialysis Membrane | 1 pkg | 78.00/pkg | 78 |
| Electrophoresis Plates | 1 pkg | 300.00/pkg | 300 |
| Plastic Scintillation Vials | 2 cases | 38.00/case | 76 |
| Glass Scintillation Vials | 2 cases | 45.00/case | 90 |
| Aquasol | 4 gal | 65.89/gal | 264 |
| Polaroid Typess Film | 6 boxes | 26.87/box | 161 |
| 35 mm Polaroid Slide Film | 20 pkg | 14.85/pkg | 297 |
| Filter Paper | 1 pkg | 25.00/pkg | 25 |
| Disposable Gloves | 10 pkg | 5.63/box | 56 |
| " " | 15 pkg | 5.82/box | 87 |
| Utility Wipes | 6 cases | 17.82/case | 107 |
| Aluminum Foil | 4 rolls | 4.08/roll | 16 |
| | TWO-DIMENSIONAL GEL ELECTROPHORESIS AND ASSOCIATED EXPENDIBLES SUBTOTAL | | $2,162 |

Fluorography Supplies

| Item | Quantity | Unit Cost | Total |
|---|---|---|---|
| Amplify | 20 l | $ 50.00/l | $1,000 |
| X-Ray Film | 2 boxes | 134.00/box | 268 |
| X-Ray Film Developer and Fixer | 25 pkg | 4.00/pkg | 100 |
| | FLUOROGRAPHY SUBTOTAL | | $1,368 |

Maintenance Contracts

| Item | Quantity | Unit Cost | Total |
|---|---|---|---|
| TM Analytic Contract for Searle Scintillation Counter | 1 year | $3,895.00/yr | $3,895 |
| International Imaging Systems Contract for Model 75 Image Processor | 1 year | 6,920.00/yr | 6,920 |
| | MAINTENANCE CONTRACT SUBTOTAL | | $10,815 |

Travel

| Item | Quantity | Unit Cost | Total |
|---|---|---|---|
| Round Trips from Oak Ridge to NIEHS | 4 | 500.00 | $2,000 |
| Scientific Meeting Allowance | 1-2 | 1,000-2,000 | 2,000 |
| | TRAVEL SUBTOTAL | | $ 4,000 |
| | GRAND TOTAL | | $24,487 |

Animals - These costs are charged monthly on the basis of Mouse Cage Equivalents (MCE's). This project proposes to use 30 MCE's per month. Based on historical data, the average cost per MCE is projected to be $28.50/month. ($28.50 x 30 MCE's/mo x 12 mos = $10,260)

Utilities - These costs are made up of charges from three sources: electricity; steam, water, and compressed air; and utility operators. Costs are distributed to projects on the basis of square foot usage of space.

INDIRECT COSTS

Laboratory Overhead - These costs are made up of two types:

General and Administrative--costs resulting from the miscellaneous business and administrative functions benefiting the laboratory as a whole (Health Division, janitors, Safety Department, fire protection, libraries, etc.).

General Plant Services--costs resulting from miscellaneous services necessary for the operation and upkeep of the entire laboratory, and not relating to a specific field function (mail services, stores, timekeeping, food services, travel, etc.).

All overhead costs from these two categories are distributed directly to specific projects on the basis of total costs less any approved exemptions.

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