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OCT 23 1980

Division of Research Grants
National Institutes of Health
Bethesda, Maryland 20205

Gentlemen:

ORNL GRANT PROPOSAL, "CHEMICAL MUTAGENESIS AND DIFFERENTIATION IN HUMAN CE
(INTERAGENCY AGREEMENT DOE NO. 40-1141-80)

Enclosed for your consideration are six copies of the subject research proposal, prepared by the DOE's Oak Ridge National Laboratory (ORNL). Also enclosed are an executed Grant Certification and an executed Cost Sharing Agreement in the format of HEW Form 490. The research objective will be to develop a series of assays, using human teratoma cells for the identification of chemical carcinogens which may initiate or promote tumor formation in experimental animals and humans.

The proposal requests a five-year period of performance beginning October 1, 1981. The initial twelve month funding will require $169,500 for direct costs.

Your favorable consideration of the proposal should be reflected in a funding authorization to this office. Upon its receipt, we will authorize ORNL to proceed with the research project. Monthly billings will be accomplished by Standard Form 1081 to reimburse the DOE appropriation for actual cost incurred.

BEST COPY AVAILABLE

REPOSITORY Oak Ridge Operations Records Holding Area
COLLECTION ~~Documents~~ 1944-94
BOX No. B-87-7 Bldg. 2714-H
FOLDER 40-1141-80 NIH

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OFFICIAL FILE COPY

Technical questions concerning the proposal should be directed to E. Huberman, ORNL Principal Investigator, telephone FTS 624-[illegible]. Administrative questions should be addressed to J. E. Carr, ORNL Work for Others Coordinator, telephone FTS 624-[illegible].

Sincerely,

Original Signed by
William R. Bibb

William R. Bibb, Director
Research Division

ER-13:WRB

Enclosures:
1. Proposal (6)
2. Grant Certification
3. Cost Sharing Agreement

cc w/encls:
W. P. Snyder, CC-10, ORO

cc w/o encls:
H. Postma, ORNL
J. E. Carr, ORNL
E. Huberman, ORNL

ER-13:WRBibb:dw:6-0742:10-21-80

CONCURRENCE
RTG. SYMBOL ~~PD-123~~ ~~Smith~~
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RTG. SYMBOL ER-13
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COST-SHARING AGREEMENT

Awarding Office:
National Institutes of Health

Application or Award Number:

Applicant:
U. S. Department of Energy
Oak Ridge Operations Office
Research Division
Post Office Box E
Oak Ridge, Tennessee 37830

Proposed Project Period:
From October 1, 1981 to September 30, 1986

ORNL RESEARCH GRANT PROPOSAL, "CHEMICAL MUTAGENESIS AND DIFFERENTIATION IN HUMAN CELLS" (INTERAGENCY AGREEMENT DOE NO. 40-1141-80)

In order to satisfy HEW cost-sharing requirements, the U. S. Department of Energy agrees to bear 17.10 percent of the total cost of the above project.

Total project cost is the sum of the total allowable direct and indirect cost incurred by the recipient and sub-recipients of cost-type contractors, plus the value of any third party in-kind contributions which benefit the project. These amounts will be calculated according to the rules in the HEW grant administration regulations at 45 CFR, Part 74.

If the project period consists of more than one budget period, the agreed-upon rate will apply to the project period as a whole, including any non-competitive extensions but not necessarily to each individual budget period.

If HEW stops funding this project prior to the close of the originally agreed-upon project period, the rate will apply to the actual period of support.

If the project period is extended competitively, another cost-sharing agreement will be negotiated.

Original Signed by
William R. Bibb

William R. Bibb, Director
Research Division

OCT 23 1980
Date

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DOE F 1325.10
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OFFICIAL FILE COPY

Certification Required by PHS: 1-515-40B

for

Chemical Mutagenesis and Differentiation in Human Cells

To Be Performed By

The Department of Energy's Oak Ridge National Laboratory

I certify that:

1. The Department of Energy has the statutory authority to carry out the research study set forth in the proposal for the research project with the above title.

2. The Department of Energy will be legally, financially and administratively responsible for the conduct of the research project with the above title if a research grant is awarded to Oak Ridge Operations of the Department of Energy for this purpose.

3. Dr. William R. Bibb, Director, Research Division, Oak Ridge Operations, is delegated by me to administer the grant for the Department of Energy.

R. J. Hart
Manager
Oak Ridge Operations
Department of Energy
OCT 23 1980

1078869

OAK RIDGE NATIONAL LABORATORY
OPERATED BY
UNION CARBIDE CORPORATION
NUCLEAR DIVISION

POST OFFICE BOX X
OAK RIDGE, TENNESSEE 37830

OFFICE OF THE DIRECTOR

October 22, 1980

Department of Energy
Oak Ridge Operations
Attention: Mr. J. A. Lenhard, Assistant Manager
for Energy Research and Development
Post Office Box E
Oak Ridge, Tennessee 37830

Gentlemen:

Research Grant Proposal to the NIH (DOE 40-1141-80), "Chemical Mutagenesis and Differentiation in Human Cells"

Enclosed for your review and approval is a research grant proposal being submitted to the National Institutes of Health. The proposal requests a five-year period of performance beginning October 1, 1981, and a total direct funding of $994,550. The initial twelve-month funding will require $169,500 plus indirect costs of approximately $52,550.

The research objective will be to develop a series of assays, using human teratoma cells for the identification of chemical carcinogens which may initiate or promote tumor formation in experimental animals and humans.

The following information is provided to assist in your review and approval of this proposal:

a. This research effort is described in the approved ORNL Institutional Plan on page 52, "Work for Others (Excluding NRC)." The personnel required for this research are included in the direct FTE's in the "Summary of Resources" on page 53.

b. E. Huberman will serve as the principal investigator devoting 25% of his effort to this research. Dr. Huberman's remaining effort will be distributed as follows: 25% to NCI (40-1142-80) and 50% to EPA(40-740-78).

c. Existing space and resources are available to conduct this research without interfering with existing DOE efforts.

1078870

40-1141-80

d. This research will serve the DOE related objectives in Activity HA 02 02 01 0, "Health Effects Research in Biomedical Systems - Carcinogenesis." Based upon this programmatic relation, it is requested that this research be conducted on an actual cost basis, depreciation and DOE added cost factor waived.

After your approval and approval of the Office of Health and Environmental Research, please forward six (6) copies to the Division of Research Grants, National Institutes of Health, Bethesda, Maryland 20205.

If you have any questions concerning this proposal, please contact C. R. Richmond, ext. 4-4332.

Sincerely yours,

C. C. Hyland
for Herman Postma
Director

HP:TV:egp

Enclosure

cc: J. E. Carr
R. J. M. Fry
R. A. Griesemer
R. F. Hibbs
E. Huberman
J. N. Maddox, DOE-GTN
W. R. Ragland
C. R. Richmond
File - RC

1078871

FORM APPROVED
O.M.B. NO. 68-R0249

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

GRANT APPLICATION

FOLLOW INSTRUCTIONS CAREFULLY

LEAVE BLANK		
TYPE	ACTIVITY	NUMBER
REVIEW GROUP		FORMERLY
COUNCIL/BOARD (Month, year)		DATE RECEIVED

1. TITLE OF APPLICATION (Do not exceed 56 typewriter spaces)
Chemical mutagenesis and differentiation in human cells.

2. RESPONSE TO SPECIFIC PROGRAM ANNOUNCEMENT ☐ NO ☐ YES (If "YES," state RFA number and/or announcement title)

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR

3a. NAME (Last, first, middle)
Huberman, Eliezer

3b. SOCIAL SECURITY NUMBER
[redacted]

3c. MAILING ADDRESS (Street, city, state, zip code)
Oak Ridge National Laboratory
Biology Division
P. O. Box Y
Oak Ridge, TN 37830

3d. POSITION TITLE
Group Leader, Carcinogenesis Program

3e. DEPARTMENT, SERVICE, LABORATORY OR EQUIVALENT
Biology Division

3f. TELEPHONE (Area code, number and extension)
615-574-0698

3g. MAJOR SUBDIVISION
None

4. HUMAN SUBJECTS, DERIVED MATERIALS OR DATA INVOLVED
[X] NO ☐ YES (If "YES," form HEW 596 required)

5. RECOMBINANT DNA RESEARCH SUBJECT TO NIH GUIDELINES
[X] NO ☐ YES

6. DATES OF ENTIRE PROPOSED PROJECT PERIOD (This application)
From: 10/1/81 Through: 9/30/86

7. TOTAL DIRECT COSTS REQUESTED FOR PROJECT PERIOD (from page 5)
$ 994,550

8. DIRECT COSTS REQUESTED FOR FIRST 12-MONTH BUDGET PERIOD (from page 4)
$ 169,500

9. PERFORMANCE SITES (Organizations and addresses)
Biology Division
Oak Ridge National Laboratory
P. O. Box Y
Oak Ridge, Tennessee 37830

10. INVENTIONS (Competing continuation application only)
Were any inventions conceived or reduced to practice during the course of the project?
[X] NO ☐ YES - Previously reported
☐ YES - Not previously reported

11. APPLICANT ORGANIZATION (Name, address, and congressional district)
Oak Ridge National Laboratory
P. O. Box Y
Oak Ridge, Tennessee 37830

Third Congressional District

12. ORGANIZATIONAL COMPONENT TO RECEIVE CREDIT FOR INSTITUTIONAL GRANT (See instructions)
Code 6 0 Description:

13. ENTITY IDENTIFICATION NUMBER
60-Department of Energy

14. TYPE OF ORGANIZATION (See instructions)
☐ Private Nonprofit
[X] Public (Specify Federal, State, Local): Federal

15. OFFICIAL IN BUSINESS OFFICE TO BE NOTIFIED IF AN AWARD IS MADE (Name, title, address and telephone number.)
William R. Bibb
Director, Research Division
Department of Energy
Oak Ridge, Tennessee 37830
FTS 626-0727; (615) 576-0727

16. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (Name, title, address and telephone number)
William R. Bibb
Director, Research Division
Department of Energy
Oak Ridge, Tennessee 37830
FTS 626-0727; (615) 576-0727

17. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

SIGNATURE OF PERSON NAMED IN 3a (In ink. "Per" signature not acceptable)
E. Huberman

DATE
10/10/80

18. CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with Public Health Service terms and conditions if a grant is awarded as the result of this application. A willfully false certification is a criminal offense. (U.S. Code, Title 18, Section 1001.)

SIGNATURE OF PERSON NAMED IN 16 (In ink. "Per" signature not acceptable)
William R. Bibb

DATE
10-20-80

PHS-398
Rev. 10/79

1018872

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: Eliezer Huberman

DETACH AND CLIP TO THE SIGNED FACE PAGE OF THE APPLICATION

PERSONAL DATA ON

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR

The Public Health Service has a continuing commitment to monitoring the operation of its review and award processes to detect—and deal appropriately with—any instances of real or apparent inequities with respect to age, sex, race, or ethnicity of the proposed principal investigator/program director.

To provide the PHS with the information it needs for this important task, the principal investigator/program director is requested to complete the form below and attach a single copy to the signed face page of the application.

Upon receipt and assignment of the application by the PHS, this form will be detached from the application. It will NOT be duplicated and will NOT be a part of the review process. Data will be confidential, and will be maintained in Privacy Act record system 09-25-0036, "Grants: IMPAC (Grant Contract Information)." All analyses conducted on the data will report aggregate statistical findings only and will not identify individuals.

If you decline to provide this information, it will in no way affect consideration of your application.

Your cooperation will be appreciated.

Date of Birth: [redacted] (Month/Day/Year)

Sex: ☐ Female ☒ Male

Race and/or Ethnic Origin:

Check one:

- ☐ American Indian or Alaskan Native
- ☐ Asian or Pacific Islander
- ☐ Black, not of Hispanic origin
- ☐ Hispanic
- ☒ White, not of Hispanic origin

NOTE: The category that most closely reflects the individual's recognition in the community should be used for purposes of reporting mixed racial and/or ethnic origins. Definitions are on the back of form.

1018813

DEPARTMENT OF HEALTH, E ATION, AND WELFARE
PUBLIC HEALT.. ERVICE

ABSTRACT OF RESEARCH PLAN

LE : BLANK

PROJECT NUMBER

NAME AND ADDRESS OF APPLICANT ORGANIZATION (Same as Item 11, page 1)
Oak Ridge National Laboratory, P. O. Box Y, Oak Ridge, Tennessee 37830

TITLE OF APPLICATION (Same as Item 1, page 1)
Chemical mutagenesis and differentiation in human cells.

Name, Title and Department of all professional personnel engaged on project, beginning with Principal Investigator/Program Director

Eliezer Huberman, Group Leader, Cancer & Toxicology Program, Biology Division
Bruce S. Hass, Research Associate, Cancer & Toxicology Program, Biology Division
Carol A. Jones, Research Associate, Cancer & Toxicology Program, Biology Division

ABSTRACT OF RESEARCH PLAN: Concisely describe the application's specific aims, methodology and long-term objectives, making reference to the scientific disciplines involved and the health-relatedness of the project. The abstract should be self-contained so that it can serve as a succinct and accurate description of the application when separated from it. DO NOT EXCEED THE SPACE PROVIDED.

We propose, by using an epithelial human teratoma cell line (P_3), to develop a set of systems for the study of environmental chemicals which may initiate and/or promote tumor formation. To accomplish this we shall undertake the following approaches: a) The human P_3 cells will be incorporated as target cells for mutagenesis studies. Resistance to 6-thioguanine and ouabain will serve as the genetic marker. Since P_3 cells do not metabolically activate many chemical carcinogens, we shall supplement the required metabolism by cocultivating the P_3 cells in a cell-mediated assay with primary rodent hepatocytes or human BY-015 carcinoma cells. b) We shall isolate P_3 cells resistant to the cytotoxic effect of various carcinogens/mutagens including N-methyl-N-nitro-N-nitrosoguanidine. These cells will be analyzed for their susceptibility to mutagenesis and for alterations in DNA repair processes by measuring changes in excision and post replication repair. c) We shall test tumor promoters, including phorbol diesters, for their effect on production and differentiation of embryoid bodies in the P_3 cultures as well as their effect on plasminogen activator and alkaline phosphatase activities. In addition, we shall determine whether they bind to cellular receptors in the P_3 cells. The susceptibility of the P_3 cells to mutagenesis and alteration in differentiation characteristics makes these human cells a unique tool for both the study and identification of environmental chemicals which may initiate and/or promote tumor formation.

LABORATORY ANIMALS INVOLVED. Identify by common names. If none, state "none"

rats, hamsters

TABLE OF CONTENTS

Number pages consecutively at the bottom throughout the application. Do not use suffixes such as 5a, 5b. Type the name of the Principal Investigator/Program Director at the top of each printed page and each continuation page.

SECTION 1. PAGE NUMBERS

Face Page, Abstract, Table of Contents	1-3
Detailed Budget for First 12 Month Budget Period	4
Budget Estimates for All Years of Support	5
Biographical Sketch-Principal Investigator/Program Director *(Not to exceed two pages)*	6-7
Other Biographical Sketches *(Not to exceed two pages for each)*	8-11
Other Support	12
Resources and Environment	13

SECTION 2.

Introduction *(Excess pages; revised and supplemental applications)*	
Research Plan	
A. Specific Aims *(Not to exceed one page)*	14
B. Significance *(Not to exceed three pages)*	14
C. Progress Report/Preliminary Studies *(Not to exceed eight pages)*	
D. Methods	15
E. Human Subjects, Derived Materials or Data	-
F. Laboratory Animals	-
G. Consultants	-
H. Consortium Arrangements or Formalized Collaborative Agreements	-
I. Literature Cited	23
Checklist	27

SECTION 3. Appendix *(Six sets) (No page numbering necessary for Appendix)*

Number of publications: _____ Number of manuscripts: _____

Other items (list):

Application Receipt Record, form PHS 3830

Form HEW 596 if Item 4, page 1, is checked "YES"

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR E. Huberman

DETAILED BUDGET FOR FIRST 12 MONTH BUDGET PERIOD
DIRECT COSTS ONLY

FROM 10/1/81 THROUGH 9/30/86

DOLLAR AMOUNT REQUESTED (Omit cents)

PERSONNEL (Applicant organization only) (See instructions)		TIME EFFORT				
NAME	TITLE OF POSITION	%	Hours per Week	SALARY *	FRINGE * BENEFITS	TOTALS*
Eliezer Huberman	Principal Investigator	25	10	12,000	2,590	14,590
Bruce S. Hass	Research Associate	75	30	36,000	7,770	43,770
Carol A. Jones	Research Associate	25	10	12,000	2,590	14,590
Catherine A. McKeown		100	40	30,200	6,500	36,700
Michael F. Callaham		50	20	15,100	3,250	18,350

* The stated numbers do not represent the actual specific salaries. ORNL uses the uniform accounting system recommended by the Federal Cost Accounting Standards Board. The uniform accounting system incorporates cost collect centers which are built around scientific and technical people performing similar research. Fringe benefits (~28%) and Division Administration (~30%) are calculated on the average cost collect center rates for scientific and technical assistance.

SUBTOTALS → 128,000

CONSULTANT COSTS (See instructions)

EQUIPMENT (Itemize)

SUPPLIES (Itemize by category)

Chemical & biochemicals	3,000	Utilities (1200 sq. ft. to be used for this project)	15,600
Cell culture glass & plasticware	9,400	Maintenance	3,000
Cell culture serum & medium	4,500		
Radiochemicals	2,000		
Animals	500		38,000

TRAVEL	DOMESTIC	1,500
	FOREIGN	
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	

ALTERATIONS AND RENOVATIONS (Itemize by category)

CONTRACTUAL OR THIRD PARTY COSTS (See instructions)

OTHER EXPENSES (Itemize by category)

Animal care	400	Reprints	500
Art work	400		
Page charge	600		
Books	100		2,000

TOTAL DIRECT COSTS (Also enter on page 1, item 8) → $169,500

PRINCIPAL INVESTIGATOR/PROGRAM DIRE R E. Huberman

BUDGET ESTIMATES FOR ALL YEARS OF SUPPORT REQUESTED
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS*		1st BUDGET PERIOD (from page 4)	ADDITIONAL YEARS SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL (Salary and fringe benefits.) (Applicant organization only)		128,000	138,250	149,300	161,250	174,150
CONSULTANT COSTS		-				
EQUIPMENT		-				
SUPPLIES		38,000	41,050	44,350	47,900	51,750
TRAVEL	DOMESTIC	1,500	1,650	1,800	1,950	2,100
	FOREIGN					
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
CONTRACTUAL OR THIRD PARTY COSTS						
OTHER EXPENSES		2,000	2,150	2,300	2,450	2,650
TOTAL DIRECT COSTS		169,500	183,100	197,750	213,550	230,650
TOTAL FOR ENTIRE PROPOSED PROJECT PERIOD (Also enter on page 1, item 7) →						$ 994,550

JUSTIFICATION (Use continuation pages if necessary): Briefly describe the specific functions of the personnel and consultants. For all years, justify any costs for which the need may not be obvious, such as equipment, foreign travel, alterations and renovations, and contractual or third party costs. For future years, justify any significant increases in any category. In addition, for COMPETING CONTINUATION applications, justify any significant increases over current level of support. If a recurring annual increase in personnel costs is anticipated, give percentage.

*The stated numbers do not represent the actual specific salaries. ORNL uses the uniform accounting system recommended by the Federal Cost Accounting Standards Board. The uniform accounting system incorporates cost collect centers which are built around scientific and technical people performing similar research. Fringe benefits (∿28%) and Division Administration (∿30%) are calculated on the average cost collect center rates for scientific and technical assistance.

PRINCIPAL ESTIGATOR/PROGRAM DIRECTOR: Eliezer Huberman

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Huberman, Eliezer	Group Leader	[redacted]

EDUCATION (Begin with baccalaureate training and include postdoctoral)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to recent representative publications, especially those most pertinent to this application. Do not exceed 2 pages.

1968	Visiting Associate, Etiology Branch, National Cnacer Institute, Bethesda, MD.
1969-1971	Postdoctorate (International Agency for Research on Cancer, WHO Fellowship) McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI.
1971	Visiting Scientist, Etiology Branch, National Cancer Institute, Bethesda, MD.
1971-1973	Scientist, Weitzmann Institute, Rehovot, Israel.
1973-1976	Senior Scientist, Weitzmann Institute, Rehovot, Israel.
1976-1980	Associate Professor, Weitzmann Institute, Rehovot, Israel.
1978-1981	Member of the National Cancer Institute's Cause and Prevention Scientific Review Committee.
1979-1980	Chairman of the Environmental Protection Agency's "Gene-Tox" Committee on Specific Gene Mutation in V79 cells.
1979-1981	Member of the National Academy of Sciences' Committee on Chemical Environmental Mutagens.
1980-	Member of the Editorial Board of Teratogenesis, Carcinogenesis, and Mutagenesis.
1976-pres.	Group Leader, Carcinogenesis Program, Biology Division, Oak Ridge National Laboratory.

Representative Publications Relevant to the Project:

1. Huberman, E. and Fogel, M. Activation of carcinogenic polycyclic hydrocarbons in polyoma-virus-transformed cells as a prerequisite for polyoma virus induction. Int. J. Cancer 15: 91-98, 1975.
2. Huberman, E. and Sachs, L. Mutability of different genetic loci in mammalian cells by metabolically activated chemical carcinogens. Proc. Natl. Acad. Sci. USA 73: 188-192, 1976.
3. Huberman, E., Sachs, L., Yang, S. K., and Gelboin, H. V. Identification of the mutagenic metabolites of benzo(a)pyrene in mammalian cells. Proc. Natl. Acad. Sci. USA 73: 607-611, 1976.
4. Huberman, E., Yamasaki, H., and Sachs, L. Independent regulation of two types of aryl hydrocarbon [benzo(a)pyrene] hydroxylase in mammalian cells. Int. J. Cancer 18: 76-83, 1976.
5. Huberman, E., Mager, R., and Sachs, L. Mutagenesis and transformation of normal cells by chemical carcinogens. Nature 264: 360-361, 1976.
6. Huberman, E. and Sachs, L. DNA binding and its relationship to carcinogenesis by different polycyclic hydrocarbons. Int. J. Cancer 19: 122-127, 1977.

7. Huberman, E. Viral antigen induction and mutability of different genetic loci by metabolically activated carcinogenic polycyclic hydrocarbons in cultured mammalian cells. In: The Origins of Human Cancer, H. H. Hiatt, J. D. Watson and J. A. Winsten, Eds., Vol. 4, Cold Spring Harbor Conferences on Cell Proliferation, pp. 1521-1535, 1977.
8. Yamasaki, H., Huberman, E., and Sachs, L. Metabolism of the carcinogenic hydrocarbon benzo(a)pyrene in human fibroblast and epithelial cells. II. Differences in metabolism to water soluble products and aryl-hydrocarbon hydroxylase activity. Int. J. Cancer 19: 378-383, 1977.
9. Langenbach, R., Freed, H. Y., and Huberman, E. Liver-mediated mutagenesis of mammalian cells with hepato carcinogens. Proc. Natl. Acad. Sci. 75: 2864-2867, 1978.
10. Huberman, E. Mutagenesis and cell transformation of mammalian cells in culture by chemical carcinogens. J. Environ. Pathol. Toxico. 2: 29-42, 1978.
11. Langenbach, R., Freed, H. Y., Raveh, D., and Huberman, E. Cell specificity in metabolic activation of the carcinogens aflatoxin B_1 and benzo(a)pyrene to mammalian cells in culture. Nature 275: 277-280, 1978.
12. Huberman, E., Chou, M. W., and Yang, S. Identification of 7,12-dimethylbenz(a)-anthracene metabolites that lead to mutagenesis in mammalian cells. Proc. Natl. Acad. Sci. USA 76: 862-866, 1979.
13. Huberman, E. and Callaham, M. Induction of terminal differentiation in human promyelocytic leukemia cells by tumor-promoting agents. Proc. Natl. Acad. Sci. 76: 1293-1297, 1979.
14. Huberman, E., Heckman, C., and Langenbach, R. Stimulation of differentiated functions in human melanoma cells by tumor-promoting agents and dimethyl sulfoxide. Cancer Res. 39: 2618-2624, 1979.
15. Langenbach, R., Malick, L., Tompa, A., Kuszynski, C., Freed, H., and Huberman, E. Maintenance of functional adult rat hepatocytes on C3H/10T1/2 cells. Cancer Res. 39: 3509-3514, 1979.
16. Jones, C. and Huberman, E. A sensitive hepatocyte-mediated assay for the metabolism of nitrosamines to mutagens for mammalian cells. Cancer Res. 40: 406-411, 1980.
17. Cabot, M. C., Welsch, C. J., Callaham, M. F. and Huberman, E. Alterations in lipid metabolism induced by 12-O-tetradecanoylphorbol-13-acetate in differentiating human myeloid leukemia cells. Cancer Res. 40: 3674-3679, 1980.
18. Hass, B., Jones, C. A. and Huberman, E. In vitro mammalian and bacterial assays for identification of potential carcinogens. In: Environmental Carcinogenesis. R. E. Olson, Ed., Plenum Press, in press.
19. Jones, C. A. and Huberman, E. The control of mammalian cell mutagenesis and di-ferentiation by chemicals which initiate or promote tumor formation. In: Biological Carcinogenesis. Marvin A. Rich and Philip Furmanski, Eds., Marcel Dekker, Inc., New York.
20. Bradley, M. O., Bhuyan, B., Francis, M. C., Langenbach, R., Peterson, A. and Huberman, E. Mutagenesis by chemical agents in V79 Chinese hamster cells: A review and analysis of the literature. Mutation Res., Reviews in Genetic Toxicology, in press.
21. Friedman, J. and Huberman, E. Post-replication and the susceptibility of Chinese hamster cells to the cytotoxic and mutagenic effect of alkylating agents. Proc. Natl. Acad. Sci. USA, in press.
22. Huberman, E., Weeks, C., Herrmann, A., Callaham, M. and Slaga, T. Alterations in polyamine levels induced by phorbol diesters and other agents that promote differentiation in human promyelocytic leukemia cells. Proc. Natl. Acad. Sci., in press.

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Bruce S. Hass	Research Associate	[redacted]

EDUCATION (Begin with baccalaureate training and include postdoctoral)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to recent representative publications, especially those most pertinent to this application. Do not exceed 2 pages.

1957	Phi Eta Sigma.
1963-1965	Research Technician, Argonne National Laboratory, Argonne, IL.
1965-1967	Scientific Associate, Laboratory of Radiation Biology, University of Texas, Austin, TX.
1967-1969	NIH Predoctoral Trainee.
1969-1970	PHS Fellow
1971	Robert A. Welch Fellow
1973	Phi Lambda Upsilon
1974-1975	Postdoctoral Fellow, Veterinary Toxicology Laboratory, Agricultural Research Service (USDA), College Station, TX (Dr. H. Mollenhauer).
1975-1978	Research Associate, Argonne National Laboratory, Argonne, IL (Dr. R.B. Webb).
1977	Sigma Xi
1978-1979	Assistant Microbiologist, Argonne National Laboratory, Argonne, IL.
1979	Who's Who in the Midwest.
1980	New York Academy of Sciences.
1979-1980	Research Associate, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN

Recent Representative Publications:

1. B. Hass. Growth functions, structural properties, and energy transfer in model hydrocarbon compounds. Dissertation Abstracts #7502859, University Microfilm, Ann Arbor, Michigan (1975).
2. B. Hass and H. G. Applegate. The effects of unsubstituted polycyclic aromatic hydroxarbons on the growth of Escherichia coli. Chemicao-Biological Interactions 10: 265-268 (1975).
3. B. Hass, T. V. Willoughby, C. N. Morimoto, D. L. Cullen, and E. F. Meyer. The solution of the structure of spirodienone by visual packing analysis. Acta Crystallographica B31: 1225-1229 (1975).
4. D. L. Cullen, B. Hass, D. G. Klunk, T. V. Willoughby, C. N. Morimoto, E. F. Meyer, G. Farges, and A. Dreiding. Structure solution by visual packing and optimization techniques. The crystal and molecular structure of spiro(5,5)undeca-1,4,7,10-tetraene-3,9-dione. Acta Crystallographica B32: 555-561 (1976).
5. H. H. Mollenhauer, B. Hass, D. J. Morré. Membrane transformations in Golgi apparatus of rat spermatids. A role for thick cisternae and two classes of coated vesicles in acrosome formation. J. Micros. Biol. Cell. 27: 33-36 (1976).
6. H. H. Mollenhauer, D. J. Morré, and B. Hass. Plasma membrane transformation in spermatogenesis revealed by aldehyde fixatives containing tannic acid. J. Ultrastructure Res. 61: 166-171 (1977).

7. B. Hass and R. M. Hedges. Energy transfer from the second excited state of spirobifluorene. Experientia 34: 320-321 (1978).
8. B. Hass and R. B. Webb. Photodynamic effects of dyes on bacteria. I. Mutagenesis of acridine orange in the dark in excision- and recombination-deficient strains of Escherichia coli. Mutation Res. 51: 279-284 (1978).
9. B. Hass and R. B. Webb. 8-Methoxypsoralen effects on survival and repair of Escherichia coli after UV irradiation: action spectra. Radiation Res. 80: 170-185 (1979).
10. B. Hass and R. B. Webb. Chemical mutagenesis by benzo(a)pyrene in Escherichia coli in the absence of any activating agents. Mutation Res. 60: 395-399 (1979).
11. B. Hass and R. B. Webb. Photodynamic effects of dyes on bacteria. III. Mutagenesis by acridine orange and 500-nm monochromatic light in repair-deficient strains of Escherichia coli. Mutation Res. 60: 1-11 (1979).
12. R. B. Webb, B. Hass, and H. E. Kubitschek. Photodynamic effects of dyes on bacteria. II. Genetic effects of broad spectrum visible light in the presence of acridine dyes and methylene blue in cheostat cultures of Escherichia coli. Mutation Res. 59: 1-13 (1979).
13. B. Hass and R. B. Webb. Photodynamic effects of dyes on bacteria. IV. Lethal effects of 460-nm and 500-nm light on DNA proficient- and deficient-strains of Escherichia coli in the presence of acridine orange. Mutation Res. (accepted).
14. B. Hass and H. E. Kubitschek. Development and application of screening tests for HYGAS process stream samples: 1978-1979. Argonne National Laboratory Report ANL-BIM, Genetic Toxicology of Coal Gasification Processes, Report No. 1 (1979).
15. H. J. Carney and B. S. Hass. Review of short-term screening tests for mutagens, toxigens, and carcinogens. Argonne National Lab oratory Report ANL-BIM-79-1 (1979).
16. M. J. Peak, B. S. Hass, and S. S. Dornfeld. The Salmonella mutagenesis assay: Reproducibility. Mutation Res. (in press).
17. B. Hass, E. E. Brooks, K. Schumann, and S. S. Dornfeld. Synergism and antagonism in mutagenic responses of Salmonella typhimurium strains TA98 and TA100 to binary mixtures of polycyclic aromatic hydrocarbons. (submitted).
18. E. Huberman, B. Hass, and C. A. Jones. In vitro mammalian and bacterial assays for identification of potential carcinogens. In: Environmental Carcinogenesis, (R. E. Olson, ed.) Marcel Dekker, Inc. (in press).

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PRINCIPAL INVESTIGATOR / PROGRAM DIRECTOR: Eliezer Huberman

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Carol Ann Jones	Research Associate	[redacted]

EDUCATION (Begin with baccalaureate training and include postdoctoral)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
[redacted]			

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to recent representative publications, especially those most pertinent to this application. Do not exceed 2 pages.

1971-1972	Research Associate, Ciba-Geigy Ltd., Basel, Switzerland (in vivo and in vitro drug metabolism studies).
1973	B.Sc. in biochemistry with 1st class honors.
1974	Science Research Council (UK) Ph.D. Research Fellowship.
1974-1978	Ph.D. Thesis; supervisor, Professor James W. Bridges. Title: Studies on the metabolic fate of some aromatic compounds in isolated cell systems.
1977	"Biorex" Postgraduate Prize.
1978-1980	Postdoctoral research fellowship; supervisor, Dr. Eliezer Huberman (development of a sensitive hepatocyte-mediated mutagenesis assay for detecting liver carcinogens).

Recent Representative Publications:

1. J. R. Fry, C. A. Jones, P. Wiebkin, P. Bellemann, and J. W. Bridges. The enzymic isolation of adult rat hepatocytes in a functional and viable state. Anal. Biochem. 71: 341-350, 1976.
2. P. Wiebkin, C. A. Jones, J. R. Fry, R. K. Lowing, and J. W. Bridges. The metabolism of biphenyl by isolated viable rat hepatocytes. Xenobiotica 6: 725-743, 1976.
3. C. A. Jones, B. P. Moore, G. M. Cohen, J. R. Fry, and J. W. Bridges. Studies on the metabolism of benzo(a)pyrene in isolated rat hepatocytes. Biochem. Pharmacol. 27: 693-702, 1978.
4. J. R. Fry, P. Wiebkin, J. Kao, C. A. Jones, J. Gwynn, and J. W. Bridges. A comparison of drug metabolizing capability in isolated rat hepatocytes and renal tubule fragments. Xenobiotica 8: 113-130, 1978.
5. P. Wiebkin, J. R. Fry, C. A. Jones, R. K. Lowing, and J. W. Bridges. Biphenyl metabolism in isolated rat hepatocytes: Effect of induction and nature of the conjugates. Biochem. Pharmacol. 27: 1899-1907, 1978.
6. C. A. Jones, B. P. Moore, G. M. Cohen, and J. W. Bridges. Metabolism of benzo(a)-pyrene to oxidative and conjugative metabolites by isolated mammalian hepatocytes. In: P. W. Jones and P. Leber (Eds.), Polynuclear Aromatic Hydrocarbons, Ann Arbor Science, Ann Arbor, Michigan, pp. 581-602, 1979.
7. J. Kao, C. A. Jones, J. R. Fry, and J. W. Bridges. Species differences in the metabolism of benzoic acid by isolated hepatocytes and kidney tubule fragments. Life Sciences 23: 1221-1228, 1978.
8. R. K. Lowing, J. R. Fry, C. A. Jones, P. Wiebkin, L. J. King, and J. W. Bridges. The early effects of chemical carcinogens on adult rat hepatocytes in primary culture. I. Quantitative changes in intracellular enzyme activities following a single dose of carcinogen. Chem.-Biol. Interact. 24: 121-131, 1979.

9. C. A. Jones and E. Huberman. A sensitive hepatocyte-mediated assay for the metabolism of nitrosamines to mutagens for mammalian cells. Cancer Res. 40: 406-411, 1980.
10. C. A. Jones, V. C. Dunkel and E. Huberman. The relationship between metabolism of nitrosamines to mutagens for mammalian cells and liver carcinogenesis. Proc. Am. Assoc. Cancer Res. 21: 304, 1980.
11. B. Hass, C. A. Jones, and E. Huberman. In vitro mammalian and bacterial assays for identification of potential carcinogens. In: R. E. Olson (Ed), Environmental Carcinogenesis, Marcel Dekker, Inc., N.Y.

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OTHER SUPPORT

(USE CONTINUATION PAGES IF NECESSARY)

For each of the professionals named on page 2, list, in three separate groups: (1) active support; (2) applications pending review and/or funding; (3) applications planned or being prepared for submission. Include all Federal, non-Federal, and institutional grant and contract support. If none, state "NONE." For each item give the source of support, identifying number, project title, name of principal investigator/program director, time or percent of effort on the project by professional named, annual direct costs, and entire period of support. (If part of a larger project, provide the titles of both the parent grant and the subproject and give the annual direct costs for each.) Briefly describe the contents of each item listed. If any of these overlap, duplicate, or are being replaced or supplemented by the present application, justify and delineate the nature and extent of the scientific and budgetary overlaps or boundaries.

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR:

(1) ACTIVE SUPPORT:

NCI YO1 CP 70222

Malignant cell transformation and mutagenesis induced by carcinogenic chemicals.
September 20, 1980 - September 19, 1982.

Principal Investigator - Eliezer Huberman* (50%)

The research involves the induction of cell differentiation in myeloid leukemia and melanoma cells after treatment with tumor promoters and the induction of mutations in Chinese hamster V79 cells after treatment with mutagens and chemical carcinogens.

Total Direct Cost $162,000

(2) EPA-DOE EPA No. 72-DX033

Energy related and environmental research.
October 1, 1980 - September 30, 1981.

Principal Investigator - Eliezer Huberman* (50%)

The research involves the study of organ specificity using various rat tissues.

Total Direct Cost $120,000

*The percent of effort of the P.I. will be adjusted in these two contracts so as to accommodate the 25% effort required in the present grant proposal.

RESOURCES AND ENVIRONMENT

FACILITIES: Mark the facilities to be used and briefly indicate their capacities, pertinent capabilities, relative proximity and extent of availability to the project. Use "other" to describe facilities at other performance sites listed in Item 9, page 1, and at sites for field studies. Using continuation pages if necessary, include a description of the nature of any collaboration with other organizations and provide further information in the RESEARCH PLAN.

[X] Laboratory: 4000 sq. ft. in addition to an animal housing facility with light-controlled (diurnal) environment; walk-in cold room; walk-in incubator room.

[ ] Clinical:

[X] Animal:

[ ] Computer:

[X] Office:

[ ] Other (_____):

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location, and pertinent capabilities of each.

4 - Bio-gard laminar flow tissue culture hoods; 3 - high efficiency fume hoods (meet NCI standards for use with chemical carcinogens); 3 - Zeiss inverted microscopies; 1 - Zeiss phase contrast microscope; 3 - binocular microscopies; Bio-Tran II automatic plate counter; Coulter counter; 8 - tissue culture incubators; Beckman spectrophotometer; Revco ultra low temperature freezer; Searle liquid scintillation counter, Model 6892. Shaking water baths, liquid nitrogen equipment, pH meters, table-top centrifuges, balances, etc. All are located in our laboratory complex in the Biology Division.

ADDITIONAL INFORMATION: Provide any other information describing the environment for the project. Identify support services such as consultants, secretarial, machine shop, and electronics shop, and the extent to which they will be available to the project.

Our laboratory has at its disposal a full-time secretary (on a half-time basis) and large, well-equipped machine and electronics shops. The shops are available for quick, responsive service calls.

A. Specific Aims

The overall aim of this proposal is to develop rapid and reliable methods for detecting chemicals in the environment which may initiate or promote tumor formation by employing a human teratoma cell system. The project as planned will be concerned with the following:

I. Development and use of human teratoma cells in assays for detecting environmental chemical mutagens and carcinogens.

II. Isolation of mutagen-resistant cells for mutagenesis and DNA repair studies.

III. Study the response of human teratoma cells to tumor promoting agents.

B. Significance

The ultimate objective of *in vitro* short-term bioassays is to predict the possible danger of chemicals in our environment that are hazardous to humans. Mutagenesis tests which employ bacteria or rodent cells as target organisms are the most commonly used (1–8). However, human cells may respond to some chemicals in the environment in a manner which is different from that of rodent cells or bacteria. This may arise from differences in cell permeability, carcinogen metabolism, or DNA repair. Thus, human cells should be more appropriate than rodent cells as target cells in such mutagenesis assays. It would be ideal to use epithelial cells, since most human malignancies are carcinomas. However, the use of human cells, especially epithelial cells, is limited. Maher *et al.* (9, 10) have studied the induction of 8-azaguanine resistant mutants in some human fibroblast strains derived from control and xeroderma pigmentosum patients. Using direct-acting mutagens such as UV and epoxides of polycyclic hydrocarbons, they achieved quantitative responses in inducing this type of mutation. However, because normal human fibroblasts exhibit low saturation density and low plating efficiency, this approach requires a large number of tissue culture vehicles, frequent medium changes, and prolonged incubation times. This obviously limits the use of such cells in screening for hazardous environmental chemicals. Normal human fibroblasts have an additional disadvantage in that they have a limited lifespan and can be subcultured for only about 50 cell generations. Thilly and his associates (11) have overcome some of the above problems by employing a human lymphoid cell line. Like many other cell lines, this lymphoid cell type lacks the enzymes required for the metabolism of many environmental chemicals, and therefore has to be supplemented with an appropriate activation system. However, since these cells grow in suspension or soft agar, they are not suitable for use in our sensitive, extensively developed cell-mediated mutagenesis assay (8, 12, 13), which requires contact between the target and metabolizing (mediating) cells (14).

To overcome these limitations, we have recently isolated a clone (P3) from a human teratoma cell line (15) which grows attached to the surface of petri dishes. These P3 teratoma cells exhibit an epithelial cell morphology and are characterized by a high cloning efficiency and by a short generation time leading to the production of large countable colonies within a short period of time. These epithelial teratoma cells are also characterized by a low spontaneous mutation frequency: for the HGPRT locus as measured by 6-thioguanine resistance. We have also found that these cells are susceptible to the mutagenic effect of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in a direct assay and to benzo(a)pyrene in the cell-mediated assay. Based on these preliminary results, we propose that these human epithelial teratoma cells be used as target cells in short-term assays (direct and cell-mediated) for detecting the mutagenic response of environmental chemicals hazardous to man.

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This mutagenic event(s) is presumably the initiation event of the carcinogenic process.

Our present state of knowledge indicates that carcinogenesis is a multistage process that may require a step or steps beyond the initial mutational event (16–19). Thus, it is possible that many environmental causative agents may act at later stages of the carcinogenesis process in a mechanism related to tumor promotion. Since certain initiating agents are presumably ubiquitous in the environment, the rate limiting determinant in cancer causation in humans may be exposure to tumor promoters and other cocarcinogens. Indeed, there are suggestions that tumor promotion is the rate limiting step in human lung carcinogenesis associated with cigarette smoking. Cigarette smoke condensates contain tumor promoters, and tobacco leaves contain a compound which resembles the phorbol esters, which are known to be classical tumor promoting agents in the mouse skin two-stage model (20,21). Recent studies suggested that compounds such as barbiturates may act as tumor promoters in experimental liver cancers (22).

Thus, it is equally important to identify environmental chemicals that are tumor promoters as it is to determine agents that are initiators of the carcinogenic process. At the present time, the in vivo tests which can be used to identify such promoting chemicals require much time and large numbers of experimental animals. It would therefore be of importance to substitute or supplement such expensive tests with simple in vitro short-term bioassays. However, most short-term in vitro assays are designed to determine mutagenic activity and therefore do not detect environmental promoting agents which may be devoid of this activity. Studies with phorbol esters, which are tumor promoters in cell cultures, have shown that a relationship can be established between the degree of promotion activity of a series of tumor promoting phorbol diesters in the mouse skin and their ability to induce plasminogen activator or to alter cell differentiation in cultured cells (20, 21, 23–30).

In view of this, we propose to develop, in addition to a human cell mutagenic assay, simple and rapid predictive tests for the study and detection of potential tumor promoting agents using the human P3 teratoma as target cells and alteration in cell differentiation and induction of plasminogen activator as the end points for detecting the activity of tumor promoting agents. In preliminary studies, we have found that the P3 cells can produce embryoid body-like structures (15,31) and that phorbol-12-myristate-13-acetate (PMA), which is a potent tumor promoting agent, can affect the development of these structures.

The use of these fast-growing human epithelial cells as target cells both for the detection of mutagens/carcinogens and for the detection of tumor promoting agents may thus represent an important step toward the ultimate development of an integrated, reliable human cell assay for both the study and the identification of chemicals potentially hazardous for humans from either the initiation or promotion aspects of tumor production.

Methods and Procedures

I. Development and use of human teratoma cells in an assay for detecting environmental chemical mutagens and carcinogens.

The ultimate goal in the risk analysis of environmental chemical carcinogens is the extrapolation of data obtained from short-term in vitro screening assays. Using human epithelial cells in such assays will make them reliable for predictions of tumor induction in human populations. We have recently isolated an epithelial cell clone (P3) from a human teratoma (15) which is characterized by a high

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cloning efficiency (65 ± 10%) and a relative short generation time (18 ± 2 hr). These properties lead to the production of large colonies within 10-12 days after seeding. The human P3 cells are also characterized by a low spontaneous mutation frequency. In the case of 6-thioguanine resistance (HGPRT locus), the mutation frequency is less than 1 per 10^7 cells. We also have found that these cells are susceptible to the direct mutagenic effect of the carcinogen/mutagen MNNG. Our preliminary results indicate that mutagenesis for 6-thioguanine resistance with this agent was dose dependent and that an optimum expression time was established at 6 - 8 days after treatment with the mutagen. Cocultivation of the P3 cells in a cell-mediated assay with hamster fibroblasts (7) in the presence of the potent carcinogen, benzo(a)pyrene, resulted in mutation frequencies which were even higher than in a comparable cell-mediated assay with the mutable Chinese hamster V79 cells. We therefore plan to use these cells to detect direct-acting chemical carcinogens, as well as those which require metabolism for their mutagenic response.

Cells. The following cell types will be used in these studies: (1) The human P3 teratoma; (2) BJ-015, human female pleural effusion from breast carcinoma (obtained from Cell Culture Laboratory, University of California, Naval Biosciences Laboratory, Naval Supply Center, Oakland, CA); (3) adult rat hepatocytes; and (4) embryonic fibroblasts. The cells will be cultured in tissue culture petri dishes in RPMI-1640, or Dulbecco's modified Eagle's medium, plus 20% fetal calf serum supplemented with penicillin (100 units/ml) and streptomycin (100 μg/ml). Cell growth will be determined, after cell dispersion with a trypsin/EDTA solution, from a hemocytometer count of the total cell number with the number of nonviable cells (as determined by trypan blue staining) subtracted.

Direct Mutagenesis Assay. To test for cellular mutation as measured by induced resistance to ouabain or 6-thioguanine, 5×10^6 P3 cells in exponential growth phase (2 days after passage) will be treated by the putative mutagenic agent in 100-mm Petri dishes for 3 h. The cells will then be dissociated with a trypsin/EDTA solution and seeded at 200 cells/60-mm dish in 5 ml of medium for determination of cloning efficiency and at 5×10^4 cells in 4 ml of medium for determination of the number of ouabain-resistant mutants. Ouabain will be added at an optimum concentration in 1 ml of medium after the appropriate expression time, presumably 2 days (7). For selection of the 6-thioguanine-resistant mutants, 10^5 P3 cells, which previously have been treated with the mutagen, will be seeded in 100-mm dishes. After an optimal expression time (which is to be determined [32, 33]), the cells will be dissociated from the growth plates and reseeded at 200 cells/60-mm dish in 5 ml of medium in order to determine cloning efficiency and at 2×10^4 cells/60-mm dish in 4 ml of medium in order to determine of the number of 6-thioguanine-resistant mutants. 6-Thioguanine will be added 2 days after reseeding at a final concentration of 40 μM in 1 ml of medium. Cloning efficiency will be determined by counting the number of Giemsa-stained colonies at 8-12 days after cell seeding, in 6-8 dishes for each point of mutagen concentration. The number of ouabain- or 6-thioguanine-resistant mutants will be determined by counting Giemsa-stained colonies at 14-18 days after cell seeding in 16-24 Petri dishes for each point of mutagen concentration. Mutation frequencies of ouabain- and 6-thioguanine-resistant mutants will be calculated per 10^6 or 10^5 survivors, based on the cloning efficiency and the number of cells seeded for mutant selection.

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Cell-Mediated Mutagenesis Assay. The human P3 teratoma cells are not metabolically capable of activating many premutagenic/precarcinogenic chemicals. These cells have to be supplemented with an exogenous metabolic activation system. In our previous studies with the mutable Chinese hamster V79 cells, we found that intact primary epithelial cells can provide the required metabolic activation step (6, 8, 13). We have named this system the cell-mediated mutagenesis assay. In this test, target cells for mutagenesis, such as V79, or in the present case, P3, are cocultivated with either primary hepatocytes or lethally irradiated embryonic fibroblasts which activate the tested chemicals and thus mutagenize the target cells. Using lethally-irradiated rodent fibroblasts as the metabolizing cells and V79 cells as the target cells, we established a relationship between the degree of mutagenicity and the degree of carcinogenicity of a series of polycyclic hydrocarbons (7). A similar relationship was established in the case of nitrosamines and aflatoxins when primary hepatocytes were used as the metabolizing cells (13, 8).

We present below a detailed protocol for the liver cell-mediated mutagenesis assay using P3 cells. When polycyclic aromatic hydrocarbons will be used, we shall include as mediating cells 5000R x-ray irradiated secondary rat, golden hamster, or human breast tumor cells each of which can efficiently metabolize these carcinogens. The following will be the procedure for the isolation of the rat hepatocytes and their use in the cell-mediated mutagenesis assay.

Male Fischer rats (80-100 g) will be standardly used in these studies. These animals are bred in the SPF facilities at ORNL and are housed in an animal room at 24° with a diurnal cycle of 12 h light and 12 h dark. Hepatocytes are isolated by the slicing method of Fry et al. (34). The rats will be killed by cervical disolocation and the liver lobes will be separately dissected out and placed in Dulbecco's phosphate buffered saline (0.5% NaCl, 0.02% KCl, 0.02% KH_2PO_4 and 0.29% $Na_2HPO_4 \cdot 12H_2O$) (PBS'A'). All solutions and media will contain 1% antibiotics and an antimycotic agent. Each lobe will be blotted dry, placed on a piece of filter paper supported by a glass plate and sliced using a microtome blade (American Optic, Buffalo, NY 14215). To obtain a high yield of cells, the slices should not exceed 0.5 mm thickness and should be cut cleanly and quickly using single strokes of the blade. The cut slices will be transferred into a 60-mm plastic Petri dish containing fresh PBS'A' and, when necessary, the slices will be teased apart using tweezers. The slices will be placed, in approximately 3 g portions, into 250 ml Ehrlenmeyer flasks containing 10 ml PBS'A' and will be shaken at 37° in a water bath (approximately 90 oscillations per min). The supernatant will be discarded and the washing procedure will be repeated twice more with fresh PBS'A'. This will be followed by two 10 minute washes in PBS'A' containing 0.5 mM EGTA to remove intercellular Ca^{++}. After the EGTA treatment, the slices will be shaken for 60 min at 37° in a mixture of 0.05% collagenase and 0.1% hyaluronidase in Hank's magnesium-free balanced salt solution containing 5 mM $CaCl_2$. The dissociated cells will be filtered through a double-layer of cheesecloth. The filtrate will be centrifuged at approximately 50 x g for 2 minutes and the pellet will be washed twice in PBS'A'. The cells finally will be suspended in Leibovitz's L-15 medium containing 2 mM glutamine, 10% fetal calf serum and antibiotics (complete L-15 medium). The viability of the cells will be determined by the exclusion of trypan blue; from our previous experience this value is usually 85-95%.

In cocultivation experiments (Fig. 1), 25-cm^2 T-flasks will be seeded with 5×10^5 (or as otherwise established) P3 cells in a 4 ml RPMI-1640 medium containing 20% fetal calf serum and 2 mM glutamine. After 18 hr, this medium will be replaced by complete L-15 medium containing 2×10^6 viable hepatocytes. Four hours later, after the hepatocytes have attached, the medium will be

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replaced with complete L-15 medium containing 25 mM Hepes buffer and the test chemical. The test chemicals will either be diluted in medium before being exposed to the cells, or when required, dissolved initially in DMSO (final concentration of DMSO in medium < 0.2%).

Eighteen hours after treatment with the chemical, the cell monolayer will be rinsed with PBS'A' and the cells dissociated in a solution (5 ml) of 0.05% trypsin and 0.2% EDTA in PBS'A'. The cells from each set of flasks (usually 3 per dose of the tested chemical) will be pooled, centrifuged, and the pellet resuspended in RPMI-1640 medium. The number of P3 cells will be determined by a haemocytometer. The P3 cells can be readily distinguished from the hepatocytes since the hepatocytes are larger and more granular in appearance. Each test compound will also be tested for mutagenicity in the P3 cells in the absence of the hepatocytes. After cocultivation, the P3 cells will be seeded in 60-mm Petri dishes in RPMI-1640 medium to determine cloning efficiencies and in 100-mm Petri dishes to determine the number of ouabain-resistant colonies.

For detection of ouabain resistant colonies, cells will be seeded at 5×10^4 per dish or as otherwise determined in 4 ml medium (15-30 dishes per dose). After 2 or more days (based on preliminary tests for expression time with the test compound), ouabain in 1 ml of medium will be added to give a final concentration of 1 μM. Ouabain resistant colonies will be stained with Giemsa and their number counted 14-16 days after cell seeding. The cloning efficiency will be determined by counting the colonies obtained in a minimum of five Petri dishes that have been inoculated 10 days earlier with 200 cells per Petri dish.

An expression time of 6-8 days (based on preliminary tests with the tested compound) probably will be required for the selection of 6-thioguanine mutants. Therefore, after dissociating the hepatocytes and P3 cells, 10^5 P3 cells will be seeded in 100-mm Petri dishes (3 Petri dishes per dose). The cells will be dissociated after 6-8 days and reseeded at 200 cells in 4 ml RPMI-1640 medium per 60-mm Petri dish for determination of cloning efficiency and at 2×10^4 cells in 4 ml of medium per 60-mm Petri dish for selection of 6-thioguanine mutants. 6-Thioguanine will be added later in 1 ml of medium at a final concentration of 30 μM. The 6-thioguanine resistant colonies will be stained with Giemsa and their number determined 12-14 days after cell seeding. Cloning efficiency will be determined by counting the number of colonies obtained 10 days after cell seeding. The mutation frequency for ouabain- or 6-thioguanine-resistance will be calculated per 10^6 survivors, based on the cloning efficiency and number of cells seeded for mutant selection. Several ouabain- and 6-thioguanine-resistant colonies will be grown for 4-6 weeks in the absence of the drugs after which the heritability of the ouabain- and 6-thioguanine-resistance will be verified through exposure of the cells to increasing concentrations of ouabain or 6-thioguanine.

In addition to hepatocytes, we will also include as metabolizing cells in the cell-mediated mutagenesis assay, x-irradiated secondary embryonic fibroblasts of rats and hamsters as well as cells from a human breast carcinoma, which we found previously to be efficient in activating benzo(a)pyrene to a mutagen for the V79 cells. Once the protocols of these systems are established, we shall analyze a series of polycyclic aromatic hydrocarbons, nitrosamines, aflatoxins, and hydrazines for mutagenic responses. Additional chemicals which are known, or are suspected of being, human carcinogens will also be tested.

II. Mutagen-resistant P3 cells for mutagenesis and DNA repair studies

Isolation

Mutant cells with altered cell susceptibility to the cytotoxic and/or mutagenic effect of chemical carcinogens mutagens may be of value in analyzing

the mechanism of repair of DNA damage caused by such chemicals. In addition, cells with enhanced susceptibility to mutagenesis may be extremely useful in improving the sensitivity of the cell-mediated mutagenesis assay to environmental chemicals.

Three methodologies are commonly used for the isolation of mutant cells. One is to allow the mutant cells, which do not replicate under imposed non-permissive conditions, to survive after the whole culture has been treated with a chemical that kills dividing cells. A typical example of this procedure is the isolation by Kao and Puck (35, 36) of auxotrophic mutants using a procedure which involves 5-bromodeoxyuridine incorporation followed by irradiation with visible light. Randtke et al. (37) has applied this technique in attempts to isolate UV-sensitive cells from Hela and Chang liver cells. However, the inherent limitation of this technique is that the mutant cells, particularly those which are sensitive to the killing agent, do not readily survive under the non-permissive conditions. In addition, this procedure requires isolation of a large number of colonies since these mutants are small in number relative to the thymidine kinase and transport mutants which result from the same selective procedure. A second procedure is the replica plating technique, like those which have greatly facilitated the isolation of bacterial mutants. At present two replica plating methods are available for mammalian cells: replica plating of cells growing in parallel in the wells of microplates (38-40), and Lederberg style replica plating using agar plate cultures (41, 42). A disadvantage of this procedure is that the required mutants must be present at very high frequencies since the replica plating itself has no selective capacity. A third method involves selecting drug resistant cells by exposing the cells to a cytotoxic agent which kills wild type cells but not the mutants. We shall use this last method because of its simplicity and our past experience with it. We have used this procedure to isolate MNNG-resistant cells from mutable Chinese hamster V79 cultures. These cells were more mutable than the parent cells at equicytotoxic doses of MNNG, and they showed an increased level of post-replication repair (43). We will attempt to isolate cell variants resistant to MNNG, ethylmethanesulfonate (EMS), and diol epoxide of benzo(a)pyrene. These cells will be useful in short-term mutagenesis assays and will allow us to study on the involvement of the various DNA repair processes on the susceptibility of human cells to the cytotoxic and mutagenic effects of environmental chemicals.

To obtain cells with an increased resistance to the cytotoxic effect of MNNG, and the other mutagens, we shall treat 10^8 P3 cells seeded at 10^7 cells per 100-mm Petri dishes with a dose of mutagen which will reduce cell survival by about 95%. After one subculture (about 5-10 days) the surviving cells will be reseeded in 4-6 Petri dishes at 5×10^6 cells per Petri dish and on the following day will be treated for 24 hr with the same dose of the mutagen. This type of treatment will be repeated 5-10 times at 1-2 week intervals, after which a series of clones will be isolated. Cells from such colonies will be tested for their chromosomal karyotype and for their susceptibility to the cytotoxic and mutagenic effect of various mutagens. Cytotoxicity and mutagenesis will be determined as described in the previous section. Cells from these colonies, which will exhibit a cell karyotype similar to the parent cells and which will demonstrate altered susceptibilities to the cytotoxic and/or mutagenic effect of the chemicals, will be used for DNA repair studies.

DNA Repair Studies

The major classes of DNA repair in mammalian cells after their treatment with chemical reactive agents are excision repair and post-replication repair

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(44–46). We shall analyze both types of repair after treatment with the mutagens.

Post-replication DNA repair will be analyzed by determination of DNA sedimentation in an alkaline sucrose gradient according to D'Ambrosio and Setlow (47). This type of DNA repair will be determined in exponentially growing cells seeded 40 hr earlier at 2×10^5 cells/60-mm Petri dish. Fresh culture medium will be added 1 hr prior to treatment with the mutagen. After 20–60 min of treatment with the mutagen, the dishes will be rinsed one time with fresh medium, and the wild-type and the variant cells will be labeled with either 1 μCi [^{14}C]thymidine per ml or 2 μCi [^{3}H]thymidine per ml. After 25 min of pulse with the labeled thymidine, the cells will be chased with 5×10^{-4} of unlabeled thymidine and 5×10^{-5}M deoxycytidine. After different chase times, the cells will be rinsed twice with cold phosphate-buffered saline, placed in 2.5 ml of ice-cold phosphate-buffered saline, and x-ray irradiated with 1500 R (48). After irradiation the cells will be incubated for 30 min at 4°C in 5 ml of trypsin solution. Then 5 ml of culture medium (to neutralize the effect of the trypsin) will be added. The cells will be centrifuged and resuspended in 0.2 ml of EDTA/NaCl (49). Aliquots of the two cell types with approximately 2000 cpm of either isotope will be combined in 50 μl (about 1.2×10^5 cells), lysed for 1 hr at room temperature in 0.2 ml of 1 M NaOH and 0.1 M EDTA solution layered on top of 5 ml of 5–20% alkaline sucrose gradient (0.1 M NaOH, 0.9 M NaCl, 0.003 Na_2 EDTA), and centrifuged at 25,000 rpm for 120 min in an SW 50.1 rotor. A 0.2-ml solution of 60% sucrose will be on the bottom of each gradient. Ten-drop fractions will be pumped off from the bottom of each tube and collected in paper strips, and the radioactivity and the weight-average molecular weight of single-strand DNA will be determined as previously described (50, 51).

Excision repair in the case of benzo(a)pyrene diol epoxide will be determined by the ratio of either [^{14}C]- or [^{3}H]-labeled carcinogen adduct and inversely-labeled thymidine parental DNA. After pretreatment of cells with labeled thymidine for 18 hr, the cells will be washed two times with fresh medium and treated for 1 hr with the labeled carcinogen. After appropriate incubation times, the medium will be removed, the cell monolayer will be washed twice with cold phosphate-buffered saline, and the cells will be dissociated with trypsin and washed three times in cold phosphate-buffered saline. The cells will be concentrated (10 to 15×10^6 cells/ml culture medium) and stored at -80° prior to extraction of the cellular DNA. After thawing, the cells will be treated with 1% sodium dodecyl sulfate, and the DNA will be isolated and purified by phenol and chloroform-isoamyl alcohol extractions as described by Brown et al. (52). RNA will be hydrolyzed by RNase treatment, and the DNA will be extracted with ethyl acetate. The specific activity of the modified DNA will be determined, followed by enzymatic hydrolysis to deoxynucleosides as described by Yamasaki et al. (53). Modified deoxyribonucleosides will be isolated by Sephadex LH-20 chromatography and further characterized on a Dupont 850 HPLC instrument by use of a Waters Bondapack C_{18} column eluted with a 30 to 60% methanol-ratio gradient at 50°, as described by Brown et al. (52).

Excision repair also will be determined by measuring the ratio of O^6-methylguanine (or ethylguanine) to that of 7-methylguanine (or ethylguanine) after treatment with labeled MNNG or EMS. The importance of the ratios of these two methylation products and the experimental methodology of the determination has been discussed by Margison et al. (54). After incubation of the cells with the labelled mutagen, the cellular DNA will be removed and dialyzed against a 0.1 standard saline citrate at 3°C. The precipitate will be obtained with 2 volumes of ethanol and then dried. The DNA is then hydrolyzed at 70°C for 30 min and the pH adjusted to 2.9 after the addition of 7-methylguanine or O^6-methylguanine as markers. The purine bases will be separated by Sephadex G-10 chromatography

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using 0.05 M ammonium formate-0.2% w/v sodium azide, pH 6.75 as eluant (flow rate 15 ml/hr). The A_{260} of the fractions will be determined with a spectrophotometer [mmoles absorption is 7.2 for guanine], the samples dried, and dissolved in hyamine (0.1 M solution in methanol). Radioactivity of the fractions then will be determined with the Texas Instrument liquid scintillation counter.

Results from these experiments will increase our knowledge of the relationship between specific types of DNA repair after carcinogen-mutagen insult and the corresponding cytotoxic and/or mutagenic event. It will also allow us to use in the cell-mediated assay human DNA repair mutants or a combination of such mutants which will be less susceptible to the cytotoxic effects of potential carcinogens but will be highly susceptible to their mutagenic effect.

III. Studies of the response of the human teratoma cells to tumor promoting agents.

This part of the proposed project is designed to study in detail the effect of PMA, a tumor promoter in the mouse skin, on the expression of various differentiation characteristics of the P3 human teratoma cells. These human cells are unique because they are useful for both mutagenesis studies and their ability to form embryoid bodies which express various differentiation characteristics (15, 31). The effect of PMA on certain parameters will be compared to those obtained with other phorbol esters and chemicals from other classes which are suspected of being tumor promoters. Tumor promoters such as the phorbol esters, unlike many chemical carcinogens, do not bind to cellular DNA. They probably initiate their effect by interacting with receptors on the cell membrane (55, 56) which is presumably followed by a downward regulation of such receptors (57, 58). It is therefore of interest, in addition to studying differentiation markers with P3 cells, to analyze the interaction of the tumor promoters with cellular receptors. Responses which will correlate with the degree of activity of tumor promoting agents from various chemical classes may be useful as tools for studying the mechanism of tumor promotion as well as for the identification of additional undiscovered tumor-promoting agents.

Evaluation of Cell Differentiation.

Cell Culture

The culture conditions for growing the P3 cells on the surface of Petri dishes as well for the determination of cell growth were presented above.

Development of Embryoid Bodies In Vitro.

Culturing the P3 cell in bacterial culture growth medium results in the formation of embryoid bodies which grow in size. We will incubate these cells in the presence of PMA and other tumor promoting agents and follow changes in morphological differentiation. At different times after treatment the cell cultures will be centrifuged and the pellets will be fixed for 2 hours in a buffered solution containing 4% formaldehyde and 1% glutaraldehyde (59). After a 30 minute rinse with cacodylate buffer 0.1M (pH 7.2) the cell pellets will be incubated overnight with 2% osmium tetroxide in 0.1 M cacodylate buffer and embedded in Epon 812. Two micron sections will be cut with a JB4 microtome and subsequently will be stained with toluidine blue and examined under the light microscope. We will follow morphological changes such as appearance of endoderm-like cells, epitheloid-like differentiation of the peripheral cells, and formation of ductal-like structures (15, 31, 60, 61).

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Assays for Plasminogen Activator

In addition to morpholoical change, we shall test, after treatment with tumor promoting agents, plasminogen activator activity which was previously shown to be associated with differentiation in teratocarcinoma and early embryogenesis (62-64). The assay for plasminogen activator in the culture medium will be performed by measuring the plasminogen-dependent fibrinolysis in multi-well dishes coated with ^{125}I-fibrin, as previously described (62, 64, 65). The growth medium will be acidified to reduce protease inhibitor activity, and 25 μl of it will be added to 0.25 ml of 0.1M tris-HCl (pH 8.1) containing 8 μg/ml of purified human plasminogen. The activity will be determined 2 hr after incubation at 37°C. Intracellular plasminogen activator will be determined after washing the culture 2 times with PBS. The scraped cells or homogenized and embryoid bodies will be extracted with a minimal volume of 0.1% Triton X-100 in water. The mixture will be centrifuged at 1000 g for 10 min and the supernatant will be assayed as described for the culture medium.

Alkaline Phosphatase

A common marker for differentiation in teratoma cells is an increased alkaline phosphatase activity (66). We will test this activity in control and treated cells. Activity will be determined as previously described (63). In this procedure the reaction mixture (0.5 ml) will consist of 0.1M glycine buffer (pH 10), 0.01M p-nitrophenyl phosphate, 0.002M $MgCl_2$ and cell homogenate. The reaction will be incubated at 37°C and will be terminated by addition of 0.5 ml of 0.75 N NaOH. One unit of alkaline phosphatase will be defined as the amount of enzyme which releases 1 μmole of p-nitrophenyl phosphate per hour. Specific activities will be given in units per mg protein. α-Foetoprotein, which increases during differentiation in some teratoma cell lines, will be assayed as described by Adamson *et al.* (67).

Binding Assay

The binding to cells of [^{3}H]-labeled tumor promoting phrobol-diesters (presumably phorbol dibutyrate due to its better solubility in liquid solution) (55, 56) and tumor promoters from non-phorbol chemical classes will be carried out in 1.5 ml high density polyethylene tubes with a pointed tip. Each tube will contain a fixed amount (30 mM) of high specific activity labeled tumor promoter, 1 - 3 x 10^6 cells in 0.25 ml RPMI-1640 (without serum). The incubation will be carried out for different times at 4° and 37°C. The reaction will be terminated by adding cold 0.5 ml PBS (pH 7.4). The tubes will be immediately centrifuged at 15,000 g for 5 min. The supernatant will be carefully removed by aspiration and the pelleted cells will be further washed with 3 x 0.5 ml of ice cold PBS (pH 7.4). Finally, the pointed tips of the tubes with the pelleted cells will be cut off and dropped into scintillation vials containing 1 ml of 1% sodium dodecyl sulfate plus 10 mM dithiothreitol. The glass vials will be left at room temperature for 2 hr at which time scintillation fluid will be added and the amount of label determined. Specific binding will be determined by the difference between the bound tumor promoter in the absence and presence of 1000 fold higher of cold material. Specific binding will be expressed as cpm bound per 10^6 cells.

After we have established the specific changes induced by tumor-promoting agents, we shall attempt to establish a relationship between the degree of cytochemical and biochemical activity in the P3 cells caused by the promoting agents and their promoting activity *in vivo*. Once this is established, we shall attempt to identify in these cells other environmental chemicals with the same characteristics. Such agents may represent potential endogenous tumor promoters.

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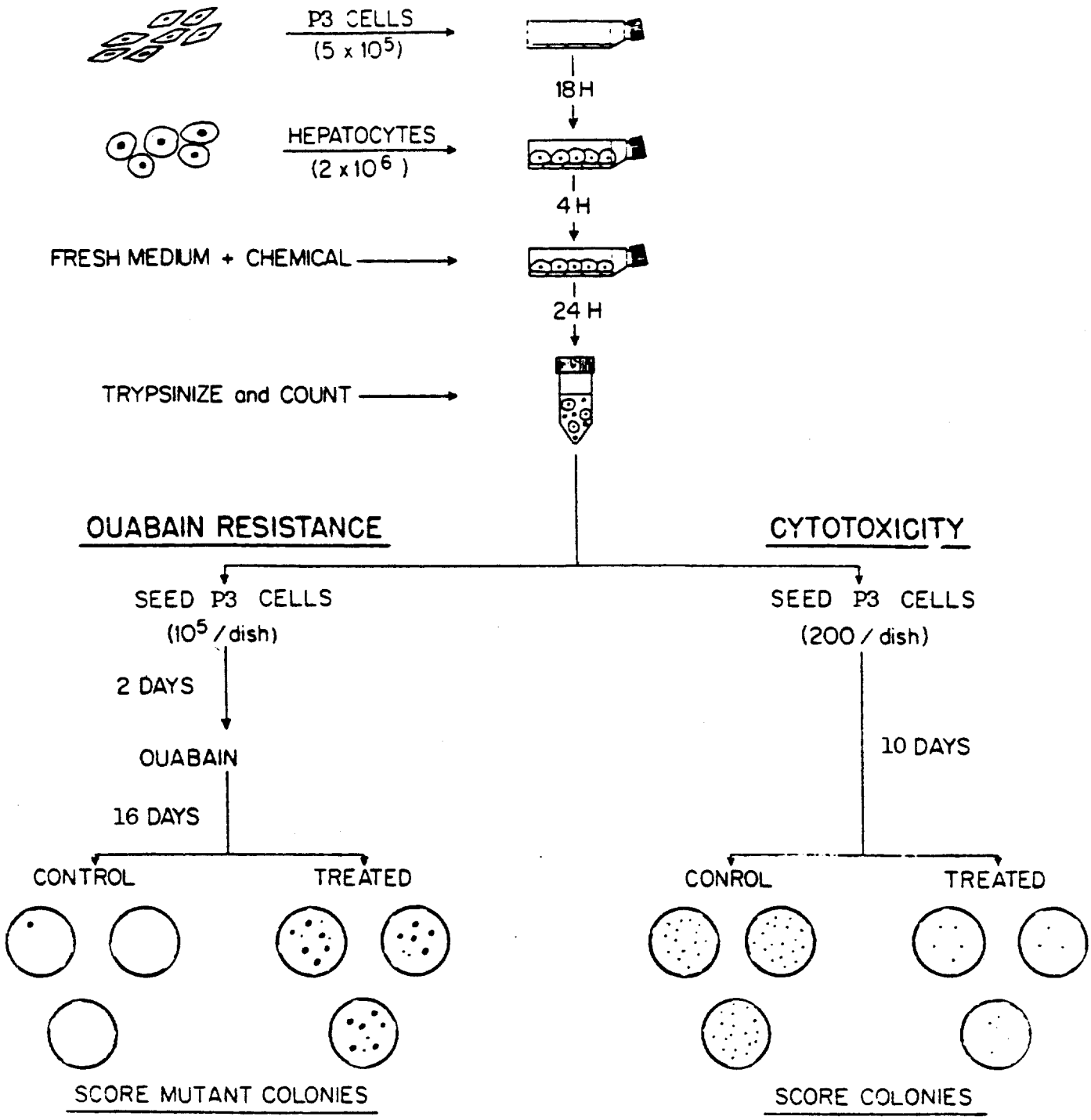
HEPATOCYTE-MEDIATED MAMMALIAN CELL MUTAGENESIS ASSAY



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CHECKLIST

This is the required last page of the application.

Check the appropriate boxes and provide the information requested.

TYPE OF APPLICATION:

[X] NEW application *(This application is being submitted to the PHS for the first time.)*

[ ] COMPETING CONTINUATION of grant number: _____.
(This application is to extend a grant beyond its original project period.)

[ ] SUPPLEMENT to grant number: _____.
(This application is for additional funds during a funded project period.)

[ ] REVISION of application number: _____.
(This application replaces a prior version of a new, competing continuation or supplemental application.)

[ ] Change of Principal Investigator/Program Director.
Name of former Principal Investigator/Program Director: _____.

ASSURANCES IN CONNECTION WITH:

Civil Rights	Handicapped Individuals	Sex Discrimination	Human Subjects General Assurance *(If applicable)*	Laboratory Animals *(If applicable)*
[X] Filed	[X] Filed	[X] Filed	[X] Filed	[X] Filed
[ ] Not filed	[ ] Not filed	[ ] Not filed	[ ] Not filed	[ ] Not filed

INDIRECT COSTS:

Indicate the applicant organization's most recent indirect cost rate established with the appropriate DHEW Regional Office. If the applicant organization is in the process of initially developing or renegotiating a rate, or has established a rate with another Federal agency, it should, immediately upon notification that an award will be made, develop a tentative indirect cost rate proposal based on its most recently completed fiscal year in accordance with the principles set forth in the pertinent *DHEW Guide for Establishing Indirect Cost Rates*, and submit it to the appropriate DHEW Regional Office. Indirect costs will not be paid on foreign grants, construction grants, and grants to individuals, and usually not on grants in support of conferences.

[X] DHEW Agreement Dated: February 14, 1979.

_____ % Salary and Wages or 35 % Total Direct Costs.

Is this an off-site or other special rate, or is more than one rate involved? [ ] YES [X] NO
Explanation: _____

[ ] DHEW Agreement being negotiated with _____ Regional Office.

[ ] No DHEW Agreement, but rate established with _____. Date _____.

[ ] No Indirect Costs Requested.

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

PROTECTION OF HUMAN SUBJECTS ASSURANCE/CERTIFICATION/DECLARATION

☐ ORIGINAL ☐ FOLLOWUP ☐ REVISION

☒ GRANT ☐ CONTRACT ☐ FELLOW ☐ OTHER

☒ NEW ☐ RENEWAL ☐ CONTINUATION

APPLICATION IDENTIFICATION NUMBER *(If known)*

STATEMENT OF POLICY: Safeguarding the rights and welfare of subjects at risk in activities supported under grants and contracts from DHEW is primarily the responsibility of the institution which receives or is accountable to DHEW for the funds awarded for the support of the activity. In order to provide for the adequate discharge of this institutional responsibility, it is the policy of DHEW that no activity involving human subjects to be supported by DHEW grants or contracts shall be undertaken unless the Institutional Review Board has reviewed and approved such activity, and the institution has submitted to DHEW a certification of such review and approval, in accordance with the requirements of Public Law 93-348, as implemented by Part 46 of Title 45 of the Code of Federal Regulations, as amended, (45 CFR 46). Administration of the DHEW policy and regulation is the responsibility of the Office for Protection from Research Risks, National Institutes of Health, Bethesda, MD 20205.

1. TITLE OF PROPOSAL OR ACTIVITY

Chemical mutagenesis and differentiation in human cells.

2. PRINCIPAL INVESTIGATOR/ACTIVITY DIRECTOR/FELLOW

Eliezer Huberman

3. DECLARATION THAT HUMAN SUBJECTS EITHER WOULD OR WOULD NOT BE INVOLVED

☒ A. NO INDIVIDUALS WHO MIGHT BE CONSIDERED HUMAN SUBJECTS, INCLUDING THOSE FROM WHOM ORGANS, TISSUES, FLUIDS, OR OTHER MATERIALS WOULD BE DERIVED, OR WHO COULD BE IDENTIFIED BY PERSONAL DATA, WOULD BE INVOLVED IN THE PROPOSED ACTIVITY. (IF NO HUMAN SUBJECTS WOULD BE INVOLVED, CHECK THIS BOX AND PROCEED TO ITEM 7. PROPOSALS DETERMINED BY THE AGENCY TO INVOLVE HUMAN SUBJECTS WILL BE RETURNED.)

☐ B. HUMAN SUBJECTS WOULD BE INVOLVED IN THE PROPOSED ACTIVITY AS EITHER: ☐ NONE OF THE FOLLOWING, OR INCLUDING: ☐ MINORS, ☐ FETUSES, ☐ ABORTUSES, ☐ PREGNANT WOMEN, ☐ PRISONERS, ☐ MENTALLY RETARDED, ☐ MENTALLY DISABLED. UNDER SECTION 6. COOPERATING INSTITUTIONS, ON REVERSE OF THIS FORM, GIVE NAME OF INSTITUTION AND NAME AND ADDRESS OF OFFICIAL(S) AUTHORIZING ACCESS TO ANY SUBJECTS IN FACILITIES NOT UNDER DIRECT CONTROL OF THE APPLICANT OR OFFERING INSTITUTION.

4. DECLARATION OF ASSURANCE STATUS/CERTIFICATION OF REVIEW

☐ A. THIS INSTITUTION HAS NOT PREVIOUSLY FILED AN ASSURANCE AND ASSURANCE IMPLEMENTING PROCEDURES FOR THE PROTECTION OF HUMAN SUBJECTS WITH THE DHEW THAT APPLIES TO THIS APPLICATION OR ACTIVITY. ASSURANCE IS HEREBY GIVEN THAT THIS INSTITUTION WILL COMPLY WITH REQUIREMENTS OF *DHEW Regulation 45 CFR 46*, THAT IT HAS ESTABLISHED AN INSTITUTIONAL REVIEW BOARD FOR THE PROTECTION OF HUMAN SUBJECTS AND, WHEN REQUESTED, WILL SUBMIT TO DHEW DOCUMENTATION AND CERTIFICATION OF SUCH REVIEWS AND PROCEDURES AS MAY BE REQUIRED FOR IMPLEMENTATION OF THIS ASSURANCE FOR THE PROPOSED PROJECT OR ACTIVITY.

☐ B. THIS INSTITUTION HAS AN APPROVED GENERAL ASSURANCE (DHEW ASSURANCE NUMBER _____) OR AN ACTIVE SPECIAL ASSURANCE FOR THIS ONGOING ACTIVITY, ON FILE WITH DHEW. THE SIGNER CERTIFIES THAT ALL ACTIVITIES IN THIS APPLICATION PROPOSING TO INVOLVE HUMAN SUBJECTS HAVE BEEN REVIEWED AND APPROVED BY THIS INSTITUTION'S INSTITUTIONAL REVIEW BOARD IN A CONVENED MEETING ON THE DATE OF _____ IN ACCORDANCE WITH THE REQUIREMENTS OF THE *Code of Federal Regulations on Protection of Human Subjects (45 CFR 46)*. THIS CERTIFICATION INCLUDES, WHEN APPLICABLE, REQUIREMENTS FOR CERTIFYING FDA STATUS FOR EACH INVESTIGATIONAL NEW DRUG TO BE USED (SEE REVERSE SIDE OF THIS FORM).

THE INSTITUTIONAL REVIEW BOARD HAS DETERMINED, AND THE INSTITUTIONAL OFFICIAL SIGNING BELOW CONCURS THAT:

EITHER ☐ HUMAN SUBJECTS WILL NOT BE AT RISK; OR ☐ HUMAN SUBJECTS WILL BE AT RISK.

5. AND 6. SEE REVERSE SIDE

7. NAME AND ADDRESS OF INSTITUTION

Biology Division, Oak Ridge National Laboratory, P. O. Box Y
Oak Ridge, TN 37830

8. TITLE OF INSTITUTIONAL OFFICIAL	TELEPHONE NUMBER
Associate Director Biomedical and Environmental Sciences	(615) 574-4333
SIGNATURE OF INSTITUTIONAL OFFICIAL Chester R. Richmond Chester R. Richmond	DATE 10/17/80

HEW-596 (Rev. 2-80)

ENCLOSE THIS FORM WITH THE PROPOSAL OR RETURN IT TO REQUESTING AGENCY.

1078900

INTRA LABORATORY CORRESPONDENCE

OAK RIDGE NATIONAL LABORATORY

August 5, 1980

To: Animal Users

From: G. A. Bingham, D.V.M. GAB

Subject: NIH Animal Use Policy

This memorandum is to assure you that the Biology Division is in compliance with the U.S. Public Health Service policy concerning the care and use of laboratory animals as prescribed in their instruction sheet for grant applications (PHS 398 Rev. 10/79). We have received their acceptance of our letter of assurance of compliance with their animal policy. In keeping with this assurance we have an active committee chaired by Dr. R. A. Popp that reviews our animal policies and operations and our animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

GAB:cr

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