Pacific Northwest Laboratory Annual Report for 1987 to the DOE Office of Energy Research

**Part 4 Physical Sciences
June 1988**

**Prepared for the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830**

**Pacific Northwest Laboratory
Operated for the U.S. Department of Energy
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L. H. Toburen

June 1988

Prepared for
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under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
Richland, Washington 99352

PREFACE

This 1987 Annual Report from Pacific Northwest Laboratory (PNL) to the U.S. Department of Energy (DOE) describes research in environment, safety and health conducted during fiscal year 1987. The report again consists of five parts, each in a separate volume.

The five parts of the report are oriented to particular segments of the PNL program. Parts 1 to 4 report on research performed for the DOE Office of Health and Environmental Research in the Office of Energy Research. Part 5 reports progress on all research performed for the Assistant Secretary for Environment, Safety and Health. In some instances, the volumes report on research funded by other DOE components or by other governmental entities under interagency agreements. Each part consists of project reports authored by scientists from several PNL research departments, reflecting the multidisciplinary nature of the research effort.

The parts of the 1987 Annual Report are:

Part 1:	Biomedical Sciences Program Manager: J.F. Park	D.L. Felton, Report Coordinator and Editor
Part 2:	Environmental Sciences Program Manager: R.E. Wildung	S.G. Weiss, Report Coordinator G.P. O'Connor, Editor
Part 3:	Atmospheric Sciences Program Manager: C.E. Elderkin	C.E. Elderkin, Report Coordinator E.L. Owczarski, Editor
Part 4:	Physical Sciences Program Manager: L.H. Toburen	L.H. Toburen, Report Coordinator P.L. Gurwell, Editor
Part 5:	Environment, Safety, Health, and Quality Assurance Program Managers: L.G. Faust B.L. Steelman J.M. Selby	L.G. Faust and B.L. Steelman, Report Coordinators S.K. Ennor, M.T. Upton, and J.M. Gephart, Editors

Activities of the scientists whose work is described in this annual report are broader in scope than the articles indicate. PNL staff have responded to numerous requests from DOE during the year for planning, for service on various task groups, and for special assistance.

Credit for this Annual Report goes to the many scientists who performed the research and wrote the individual project reports, to the program managers who directed the research and coordinated the technical progress reports, to the editors who edited the individual project reports and assembled the five parts, and to Ray Baalman, editor in chief, who directed the total effort.

Members of the Scientific Advisory Committee, established in 1985, are:

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W.J. Bair, Manager
Environment, Health and Safety
Research Program

Previous reports in this series:

Annual Report for

1951	HW-25021, HW-25709
1952	HW-27814, HW-28636
1953	HW-30437, HW-30464
1954	HW-30306, HW-33128, HW-35905, HW-35917
1955	HW-39558, HW-41315, HW-41500
1956	HW-47500
1957	HW-53500
1958	HW-59500
1959	HW-63824, HW-65500
1960	HW-69500, HW-70050
1961	HW-72500, HW-73337
1962	HW-76000, HW-77609
1963	HW-80500, HW-81746
1964	BNWL-122
1965	BNWL-280, BNWL-235, Vol. 1-4; BNWL-361
1966	BNWL-480, Vol. 1; BNWL-481, Vol. 2, Pt. 1-4
1967	BNWL-714, Vol. 1; BNWL-715, Vol. 2, Pt. 1-4
1968	BNWL-1050, Vol. 1, Pt. 1-2; BNWL-1051, Vol. 2, Pt. 1-3
1969	BNWL-1306, Vol. 1, Pt. 1-2; BNWL-1307, Vol. 2, Pt. 1-3
1970	BNWL-1550, Vol. 1, Pt. 1-2; BNWL-1551, Vol. 2, Pt. 1-2
1971	BNWL-1650, Vol. 1, Pt. 1-2; BNWL-1651, Vol. 2, Pt. 1-2
1972	BNWL-1750, Vol. 1, Pt. 1-2; BNWL-1751, Vol. 2, Pt. 1-2
1973	BNWL-1850, Pt. 1-4
1974	BNWL-1950, Pt. 1-4
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1976	BNWL-2100, Pt. 1-5
1977	PNL-2500, Pt. 1-5
1978	PNL-2850, Pt. 1-5
1979	PNL-3300, Pt. 1-5
1980	PNL-3700, Pt. 1-5
1981	PNL-4100, Pt. 1-5
1982	PNL-4600, Pt. 1-5
1983	PNL-5000, Pt. 1-5
1984	PNL-5500, Pt. 1-5
1985	PNL-5750, Pt. 1-5
1986	PNL-6100, Pt. 1-5

FOREWORD

Part 4 of the Pacific Northwest Laboratory Annual Report for 1988 to the DOE Office of Energy Research includes those programs funded under the title "Physical and Technological Research." The Field Task Program Studies reports in this document are grouped by budget category and each section is introduced by an abstract that indicates the Field Task Proposal/Agreement reported in that section. These reports only briefly indicate progress made during 1987. The reader should contact the principal investigators named or examine the publications cited for more details.

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Analytical Studies

• DNA Adducts as Indicators of Health Risks

The objective of this program is to develop specific analytical methods for the determination of adducts formed in mammals by the reaction of carcinogenic compounds with cellular DNA. DNA adducts are closely associated with the formation of cancerous cells, and their concentrations are thought to be related to the amount of exposure to carcinogenic chemicals. This program is developing mass spectrometric methods for analysis of adducts that may be applied toward health studies of human exposure to carcinogens.

DETERMINATION OF ADDUCTS OF POLYCYCLIC AROMATIC HYDROCARBONS TO DNA

R. M. Bean, E. K. Chess, B. L. Thomas, D. A. Dankovic, J. Pavlovich,* D. B. Mann, J. A. Franz, and D. L. Springer

Recent studies have correlated the carcinogenic potential of certain organic compounds with the ability of their metabolites to form chemical bonds with deoxyribonucleic acids (DNA). This suggests that analysis of DNA for bound metabolites can provide an estimate of individual exposure to carcinogens. Although several techniques have recently evolved for determining total DNA binding, general methods are not available for identifying the bound organic compounds because of the very low concentrations involved. Methods currently being used for analysis of DNA adducts have recently been reviewed by Wogan and Gorelick (1985). In general, the currently available methods suffer either from a lack of sufficient sensitivity for environmental screening or from a lack of qualitative specificity. Everson et al. (1986) reported detecting concentrations of an individual adduct in the placenta of human cigarette smokers as low as 1 adduct per 10^{10} DNA base units, corresponding to one adduct per cell. Although data on humans is sparse, we may expect, allowing for initial rapid decay after exposure, adduct levels of 0.1 to 0.01 nanograms per milligram of DNA. The objective of this work, first funded as a separate DOE program in FY 1987, is to develop methods for the analysis of DNA adducts that will permit identification and quantification of adducted polycyclic aromatic hydrocarbon metabolites at environmental levels. The primary analytical approach is to isolate the bound metabolites by cleaving the covalent bonds that attach them to the DNA, and to treat them with reagents that will increase their sensitivity to mass spectrometric detection.

*Oregon State University (NORCUS student).

One of the limitations inherent in the direct analysis of adducts has been the lack of availability of analytical standards. In initial experiments, we prepared nucleotide adducts of benzo[a]pyrene (B[a]P) in nanogram quantities with calf thymus DNA, using microsomal preparations as the enzyme system. The method was found to be tedious, produced many experimental artifacts, and was incapable of producing adducts in the quantities required (micrograms) for analytical study. Since the preparation of adducts in quantity from nonradiolabeled substrates using biological systems is a vital step in this study, we have searched for a more suitable method.

D. A. Dankovic and D. L. Springer, investigators on a related program, Complex Mixture Biostudies, have found that when DNA and B[a]P were exposed to freshly isolated hepatocytes, 1 microgram of the expected adduct structure was recovered (Dankovic et al. 1987). The method is probably capable of producing several times as much when optimized. The significance of this result is that for the first time, research quantities of many different adducts can be prepared from adducting hydrocarbons and pure DNA. Research quantities of adducts have until now been prepared by synthesizing the expected diol epoxide and directly reacting this with DNA. Not only is this expensive, but one has to presume the structure of the diol epoxide, make the adduct, and compare with an adduct of biological origin to ensure that the presumption was correct.

During the course of developing an analytical method, it was found that the acid hydrolysis conditions used (80°C , 6 h, 0.12 N HCl) for the removal of the B[a]P adduct from DNA (Rahn et al. 1982) also caused the rearrangement of the resulting tetrol to an equilibrium distribution of isomers. The liquid chromatographic retention times indicated that isomerization was a result of hydroxyl epimerization about the 10-position. This has been confirmed from nuclear magnetic resonance (NMR) studies (Figure 1).

FIGURE 1. NMR Confirmation of C10 Epimerization

Nuclear magnetic resonance spectra of authentic standards were compared to isomerates collected after liquid chromatographic separation, and confirmed that the isomerization involved the 10-position only. Using molecular mechanics models, calculations of the heats of formation of cations at both the 7- and the 10-position have shown that the 10-position cation is 12.6 kcal lower (124.6 versus 137.2). This explains why no isomerization occurs at the seemingly equivalent 7-position. Recoveries of acid hydrolyzates are 71% to 88%, depending on the starting isomer. This work has shown that it is not possible to determine the exact stereo chemistry of the adducts through analysis of their acid hydrolysis products, as suggested by Shugart, Holland, and Rahn (1983). These results are being prepared for publication.

We have also continued to explore the properties of derivatives of B[a]P tetrols. In our analytical scheme, the preparation of

derivatives is a necessary step in preparing the adducting metabolites for analysis. Derivatizations of B[a]P metabolites that have one, two, three and four hydroxyl groups have been studied. The mass spectrometry of the permethyl, peracetyl, and pertrifluoroacetyl derivatives of the B[a]P metabolites has been studied in some depth, in order to understand the fragmentation patterns and thereby enable prediction of fragmentation patterns of other polycyclic aromatic hydrocarbon metabolites. Electron impact and positive and negative chemical ionization mass spectrometric studies have been conducted. The results have been accepted for publication in *Biomedical and Environmental Mass Spectrometry*. Although some fragmentations could be predicted *a priori*, other important cleavages were not predicted, and could only be conclusively established through high-resolution secondary fragmentation experiments. The permethyl triol and permethyl tetrol undergo a reverse Diels-Alder cleavage. From the cleavage patterns

determined with the B[a]P tetrol derivatives, cleavages of chrysene and dibenzanthracene tetrols were accurately predicted.

An intensive effort has been made this year to develop analytical preparation procedures that will result in the isolation of polycyclic aromatic hydrocarbon (PAH) metabolite analytes relatively free from interfering contaminants. Although the sensitivity of our analysis by selected ion mass spectrometry is less than 1 picogram, the sample must be free from interfering material for analysis of samples at that level. Sample cleanup is a necessary and often difficult task in trace analysis; however, at the levels required for DNA adduct analysis, the problem is greatly magnified. Techniques found to be perfectly adequate at the nanogram level are ineffective at the picogram level. Normal procedures for following sample losses during analysis, such as the use of radiolabeled compounds, have not proven to be effective, and a good deal of empirical experimentation has been conducted to identify sources of contamination and sample loss. Sources of contamination have been found to arise from solvents, glassware, evaporation gases, chemicals used in DNA isolation, resins used for separation, and derivatizing reagents. All of these are also potential sources of sample loss. We have made good progress in isolating and eliminating analytical problems from many of these sources.

Shown in Table 1 are the results of a recovery study from the methylation of various quantities of B[a]P tetrol. In general, the study demonstrates that reasonable recoveries of material free from interference are obtained at levels approaching those required; however, at the lowest level investigated, recoveries are considerably in excess of starting concentrations. We believe that this is a result of gas chromatographic phenomena. After analysis of several samples, the apparent sensitivity of our mass selective detector to standards increases.

TABLE 1. Mass Spectrometric Determination of Benzo[a]pyrene Tetrol Standards by Methylation and Gas Chromatography/Mass-Spectrometric Detection (GC/MSD) Analysis

Sample Quantity	Recovery, %
700 ng	85 ± 5
550 pg	115 ± 5
138 pg	113 ± 1
55 pg	99 ± 44
14 pg	248 ± 124

There is some deterioration of peak shape as well. It is likely that constituents in the sample matrix deactivate the column, allowing more of the analyte to pass through the column in later analyses than in earlier analyses. This result indicates that continued method development will involve studies of chromatographic column inertness and reproducibility.

This project is supporting the work of Dr. Barofsky of Oregon State University (OSU), through a NORCUS graduate student appointment. The student, J. Pavlovich, conducted studies at Pacific Northwest Laboratory (PNL) this summer to learn adduct analysis techniques developed here. He will do the bulk of his thesis research at OSU. The student project, conducted jointly at OSU and PNL, will involve development of ultra-sensitive mass spectral methods for analysis of DNA adducts. In preliminary studies, Dr. Barofsky has used a focused metal ion beam to detect organic compounds at the 100-picogram level, an improvement in sensitivity of about one million-fold over similar secondary ion mass spectrometric techniques.

In addition to studies related to dosimetry of hydrocarbon/DNA adducts, the PNL adduct analytical team is also engaged in an Exploratory Research Project to identify forms of hydrocarbon adducts that do not exhibit the behavior of adducts formed through normal diolepoxide addition to DNA bases. A number of researchers (e.g., Shen, Fahl, and Jefcoate 1980; Ashurst and Cohen 1982) have reported that when radiolabeled B[a]P adducts are isolated by the conventional method (enzymatic hydrolysis of DNA to the adducted nucleosides and adsorption of the adducts on LH-20), 1/3 to 1/2 of the radiolabeled nucleoside material is not retained on the LH-20. We have examined this "non-classical" adduct material by treating it with 0.12 N HCl in an attempt to release it from the residual DNA structure, followed by solid reverse-phase adsorption of the hydrolyzed product. Elution of the reverse-phase resin with methanol resulted in isolation of 66% of the original non-classical radioactivity. Reverse phase chromatography of this material revealed that in addition to tetrols, from 10% to 33% had retention times consistent with B[a]P-3, 6-quinone and B[a]P-6,12-quinone. Because "classical" B[a]P-adducted nucleosides, B[a]P tetrols, and B[a]P quinones are strongly retained on LH-20, it is apparent that the tetrols and quinones were released from the non-classical adducts during the acid hydrolysis step. We believe that determination of the

structure of the adducted material from which the quinones are derived is important; the quinones comprise as much as 10% of the radioactivity in the isolated DNA.

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• Chernobyl Data Base Management

The purpose of this project is to design and maintain an information management system to serve as the United States repository for information related to the Chernobyl nuclear accident of April 1986. The technical orientation of the project is information management to provide data for study by other scientists.

The information management system provides storage and retrieval of radiological sample results and supporting data. Supporting data include names of organizations reporting data, data collection/analysis procedures, meteorology, etc., as well as a bibliography of journal articles and reports from which data were extracted. Additionally, bibliographic information is available for numerous reports and journal articles that do not contain sample results. Information is distributed to researchers upon request for various scientific studies, including source term characterization, atmospheric transport and deposition, atmospheric concentrations, human exposure from deposited debris, human exposure via the food chain, long-term health effects/epidemiological studies, and model validation.

CHERNOBYL DATA BASE

F. Carr, Jr., J. S. Littlefield,
D. E. Robertson, W. E. Davis, E. H. Palmer,
A. D. McKinnon, L. L. Mehlenbacher, and
J. M. McClelland

Most of the work performed this past year has involved designing a relational data base structure to manage the data and obtaining data to load into the structure. Additional work has been performed in standardizing emergency response data and responding to requests for data from other organizations.

The data base structure has been designed using structured systems design techniques that include use of a data dictionary and data flow diagrams. The structure has been implemented using Digital Equipment Corporation's Rdb, a relational data base management system. Signal Technology's Smartstar application design system is being used to create the programmer/user interface to the Chernobyl data base system.

Two major sets of data have been loaded into the relational data base. They are the U.S. Environmental Protection Agency's (EPA) Domestic Data and the Department of Social and Health Services' (DSHS) data from Washington State. Tables 1 through 6 show these sets of data classified by geographical location, media, and isotope, with numbers of samples for each category. Tables 1

through 3 summarize the EPA data; Tables 4 through 6, the DSHS data.

A third major set of data, collected by EG&G in response to the Chernobyl emergency, has been entered into the computer in an interim data base, but has not been loaded into the relational data base. In addition, available supporting data have been added from the source documents. The diversity of these data requires additional work to ensure compatibility with other data and to ensure standardization. Geographical locations, media, and isotopes for this set of data with the sample quantities of each class are shown in Tables 7 through 9.

Aside from the data already entered into the computer, several other sets of data have been obtained. The majority of the data will be loaded into the data base early in 1988. A list of the larger sets of data appears in Table 10. This table shows the organization supplying the data, the estimated number of sample results, the date the data was received (or date expected if not yet received), and a short description of the data.

In addition to loading data into the system to populate the data structure, two organizations have requested data from the information management system. The requesting organization, the date the request was filled, and a brief description of the request is shown in Table 11.

TABLE 1. State Breakdown for EPA Domestic Data

<u>State</u>	<u>Number of Samples</u>
AK	95
AL	281
AR	109
AZ	70
CA	223
CO	118
CT	95
DC	16
DF	76
FL	205
GA	67
HI	88
IA	89
ID	174
IL	96
IN	95
IS	1
KS	93
KY	63
LA	48
MA	62
MD	39
ME	98
MI	116
MN	130
MO	119
MS	109
MT	65
NC	149
ND	101
NF	134
NH	108
NJ	114
NM	98
NV	92
NY	407
OH	289
OK	95
OR	102
PA	369
PC	13
PR	21
RI	71
SC	117
SD	84
TN	217
TX	193
UT	67
VA	116
VI	1
VT	72
WA	191
WI	97
WV	71
WY	71
Unknown	3

TABLE 2. Media Breakdown for EPA Domestic Data

<u>Media</u>	<u>Number of Samples</u>
Air	3706
Animal/Milk	1300
Other	7
Water/Ground	179
Water/Rain	748
Water/Surface	2
Water/Other	359
Unknown	2

TABLE 3. Isotope Breakdown for EPA Domestic Data

<u>Isotope</u>	<u>Number of Samples</u>
All ^(a)	400
Alpha	195
Beta/Gamma	4355
Am-241	1
Ba-140	1400
Be-7	4
Bi-214	258
Ce-144	250
Co-60	257
Cr-51	6
Cs-134	210
Cs-136	121
Cs-137	1531
H-3	475
I-131	1821
I-132	28
K-40	1291
La-140	157
Pb-210	20
Pb-214	9
Po-210	20
Pu-238	24
Pu-239	24
Ra-226	128
Ra-228	109
Rn-222	358
Ru-103	281
Ru-106	396
Sr-89	472
Sr-90	472
Th-227	49
Th-228	49
Th-230	49
Th-232	304
U-234	125
U-235	125
U-238	127
Zn-65	256
Zr-95	2
Zr-Nb	250

(a) Specified as analyzed for "all" radioisotopes.

TABLE 4. City and/or County Breakdown for Washington State DSHS Data

City or County	Number of Samples
Aberdeen	10
Bellingham	7
Camas	7
Cashmere	3
Castle Rock	15
Cedar River	1
College Place	7
Colville	1
Concrete	1
Deer Harbor	1
Deer Park	4
Elma	5
Forks	1
Grays Harbour	12
Hoquiam	13
Jefferson County	1
Lopez	3
Mount Vernon	4
Pacific Beach	1
Park	1
Pasco	3
Pierce County	15
Port Angeles	14
Port Townsend	16
Portland	2
Richland	11
Seattle	26
Skagit County	15
Spokane	27
Sumner	5
Sunnyside	17
Tacoma	13
Tolt Headwater	1
Tonasket	3
Tumwater	83
Vancouver	6
Walla Walla	30
Wapato	13
Wenatchee	2
Yakima	21
Yakima County	1
Unknown	32

TABLE 5. Media Breakdown for Washington State DSHS Data

Media	Number of Samples
Air	88
Animal/Milk	137
Vegetation/Other	2
Vegetation/Alfalfa	10
Vegetation/Apples	1
Vegetation/Asparagus	14
Vegetation/Broccoli Leaves	1
Vegetation/Cabbage	1
Vegetation/Corn	1
Vegetation/Garden Vegetable Composite	1
Vegetation/Grass	1
Vegetation/Green Onions	3
Vegetation/Leafy Garden Composite	1
Vegetation/Lettuce	4
Vegetation/Mixed Vegetables	1
Vegetation/Parsley	2
Vegetation/Pasture	3
Vegetation/Pasture Grass	8
Vegetation/Pasture Grass and Clover	2
Vegetation/Pears	1
Vegetation/Radish	3
Vegetation/Rhubarb	6
Vegetation/Spearmint	4
Vegetation/Spinach	13
Vegetation/Tomatoes	1
Water/Other	1
Water/Drinking	88
Water/Rain	53
Water/Standing	3

TABLE 6. Isotopes for Washington State DSHS Data

Isotope	Number of Samples
Alpha	34
Beta/Gamma	83
Ba-140	8
Be-7	25
Cd-109	1
Cs-134	163
Cs-137	242
H-3	13
I-131	435
K-40	9
Ru-103	110
Ru-106	1
Sr-89	28
Sr-90	27
Te-132	3
Th-234	2

TABLE 7. Country Breakdown for EG&G Data

<u>Country</u>	<u>Number of Samples</u>
Africa	2
Arctic Circle	4
Arctic Ocean	4
Atlantic	5
Austria	846
Beaufort Sea	1
Belgium	38
Bulgaria	6
Canada	4
Czechoslovakia	28
Denmark	39
Europe	30
Finland	1120
France	40
Germany FR	495
Germany GDR	57
Greece	44
Greenland	7
Hungary	220
Incirlik	1
Ireland	6
Italy	195
Japan	81
Luxembourg	6
Mediterranean	21
Moscow	1
Netherlands	116
North Sea	31
Norway	131
Norwegian Sea	50
Pacific	31
Poland	229
Portugal	7
Romania	16
Rumania	12
Sweden	99
Scotland	2
Siaber Sea	1
South Korea	10
Sweden	1285
Switzerland	110
Turkey	48
Britain	369
USA	277
USSR	852
USSR/Poland	1
Yugoslavia	2
Unknown	87

TABLE 8. Media Breakdown for EG&G Data

<u>Media</u>	<u>Number of Samples</u>
Air	3895
Background	1
Clothing	8
Contamination	3
Egg	1
Fish	1
Grass	68
Ground	1176
Lab	2
Meat	17
Milk	473
Rain	16
Scan	5
Smear	1
Soil	1
Spectra	1
Thyroid	95
Vegetable	172
Water	324
Weather	147
Whole Body	575
Other	85

TABLE 9. Isotopes for EG&G Data

<u>Isotope</u>	<u>Number of Samples</u>	<u>Isotope</u>	<u>Number of Samples</u>
Alpha	3	I-133p	1
Beta	5	I-136	3
Beta/Gamma	16	Kr	4
Gamma	27	Ia-140	68
Ag-110	1	Mn-54	7
Ag-110m	1	Mo-99	30
Ba-140	136	Nb-95	29
Ba-La	1	Nd	2
Ba-La-140	18	Np-239	62
Ba-La-144	1	Pb-212	1
Be-7	3	Ra-140	1
Bi-212	1	Rh-105	2
C-14	5	Rh-106	2
Cd-115m	1	Ru-103	249
Ce-131	1	Ru-106	25
Ce-141	26	Sb-122	5
Ce-144	12	Sb-125	2
Co-58	41	Sb-127	3
Co-60	14	Sr-85	1
Cr-51	2	Sr-90	1
Cs-132	11	Tc-99m	24
Cs-134	257	Tc-Mo-99m	12
Cs-136	105	Te-129	3
Cs-137	468	Te-129m	2
Fe-59	7	Te-131	11
H-3	5	Te-132	248
I	16	Tl-208	1
I-131	1778	U	1
I-131g	14	Xe	45
I-131p	38	Xe-133	6
I-131t	3	Zn-65	7
I-132	86	Zr-95	59
I-132g	6	Zr-Nb	1
I-132p	11	Zr-Nb-95	1
I-133	106	Other	2922

TABLE 10. Chernobyl Data to Be Added to the Data Base Reports Containing 1000 or More Sample Results

<u>Supplier</u>	<u>Approximate Number of Records</u>	<u>Date Received/ Expected</u>	<u>Description</u>
Brookhaven National Lab	1,000	07/01/87	Whole body/thyroid counts
Institut de Protection et de Surete Nucleaire (CEA)	10,000	11/15/87	Largely French data
Health and Welfare Canada	5,000	11/30/87	Canadian data—milk, precipitation, air samples
Stone and Webster	5,000	06/01/87	U.S. data—various media and isotopes
Environmental Measurements Laboratory	5,000	11/15/87	Mostly U.S. data, some foreign— various media and isotopes
Environmental Protection Agency	5,000	06/22/87	European data—rain, air, vegetation, milk, deposition, and experimental samples
Nuclear Regulatory Commission	1,000	07/29/87	U.S. data—vegetation, milk, air, precipitation, samples
	1,000	10/31/87	European data—air, rain, forage, and milk samples

TABLE 11. Chernobyl Data Base Information Requests

1. International Atomic Energy Agency Date Filled: 07/23/87
 Provided U.S. data including
 Date: 04/22/86 - 05/14/86
 Media: air, ground
 Isotopes: gross beta, I-131, Cs-137
2. Nuclear Regulatory Commission (Region 1) Date Filled: 08/11/87
 Provided all data within a 100-mile radius of the Chernobyl accident sorted by country, city, media, and date.

• Supercritical Fluid Analytical Methods

Supercritical fluids are providing the basis for new and improved methods across a range of analytical technologies. New methods are being developed to allow the detection and measurement of compounds that are incompatible with conventional analytical methodologies. Characterization of process and effluent streams for synfuel plants requires instruments capable of detecting and measuring high-molecular-weight compounds, polar compounds, or other materials that are generally difficult to analyze. The purpose of this program is to develop and apply new supercritical fluid techniques for extraction, separation, and analysis. These new technologies will be applied to previously intractable synfuel process materials and to complex mixtures resulting from their interaction with environmental and biological systems.

SUPERCRITICAL FLUID ANALYTICAL METHODS

R. D. Smith, H. T. Kalinoski, B. W. Wright,
and H. R. Udseth

Chromatographic methods combined with mass spectrometry (MS) compose a nearly ideal analytical approach for characterizing complex mixtures. At Pacific Northwest Laboratory (PNL), supercritical fluid methods are being developed for materials where conventional gas or liquid chromatographic (LC) methods and existing mass spectrometric methods are inadequate (see references following this article). The advantages of supercritical fluid chromatography (SFC) accrue from the nature of supercritical fluids. The solvating properties of supercritical fluid mobile phases approach those of liquid phases, yet solute diffusivity typically remains one to two orders of magnitude higher. These properties allow high separation efficiencies to be realized while exploiting the variable and often high solvating power of the supercritical fluid. Consequently, SFC can separate thermally labile and nonvolatile solutes [a procedure not possible with gas chromatography (GC)] and can do so with higher efficiencies.

The greatest immediate area of applicability of SFC-MS is for mixtures containing moderately polar compounds that are thermally labile or of relatively high molecular weight. The solvating powers available with supercritical fluids effectively "extend" the applicability of GC, so as to allow separations difficult by LC (e.g., moderate to high molecular weight polymers) or GC due to insufficient volatility or lability. On the basis of chromatographic resolution, GC, where applicable, should always be the method of choice. Similarly, given a choice between LC and SFC, SFC should generally provide superior separations. In terms of sensitivity, similar considerations are also relevant (given the same chromatographic column and ionization process) because to a

reasonable approximation, analyte molar concentration in the mobile phase (liquid, supercritical fluid, or gas) will be inversely proportional to the density of the mobile phase and directly proportional to peak width. Thus, both chromatographic resolution and sensitivity are generally expected to be in the order $GC > SFC > high\text{-}pressure\ liquid\ chromatography\ (HPLC)$. Either chromatographic resolution or detection sensitivity can usually be improved at the expense of the other.

Along with superior separations, in comparison with HPLC, SFC also offers advantages for interfacing with MS. Our early work with capillary SFC-MS has demonstrated that both fast and high resolution separations can be obtained. More recently, several other laboratories have demonstrated a range of SFC-MS applications and produced results superior to LC-MS in a number of cases. The added flexibility to choose ionization modes [electron ionization, chemical ionization (CI) with nearly any reagent gas and atmospheric pressure ionization] increases the amount of information that can be obtained by SFC-MS. This flexibility can be compared with thermospray LC-MS, where mass spectra often show negligible contributions from fragment ions from which structural information can be derived.

Although high resolution separations with capillary SFC-MS have been well demonstrated, truly useful SFC-MS instrumentation must also address more mundane problems related to sample injection methods and quantification. Nearly all capillary SFC separations reported to date with small diameter (<100- μm i.d.) capillaries have used split injection techniques, which can have low reproducibility and result in constraints on sample size because of the small injection volume and the often limited analyte solubility in the solvent. This is further complicated by the relatively small sample size typically required to avoid overloading such

columns (usually on the order of 10 ng per component for 50- μ m-i.d. columns with a 0.25- μ m film thickness) caused by the low phase ratios of such columns. While higher separation efficiencies are obtained with capillary columns, higher sample loadings are achievable with packed columns because of the greater phase ratio (relative volume of stationary phase compared to mobile phase), allowing improved detection limits. Thus, there is the continued interest in conventional (LC type) packed and microbore columns for SFC.

An area of particular concern in the SFC-MS interface is the fluid depressurization through the flow (pressure) restrictor. Application of restrictors is limited by solute precipitation for nonvolatile analytes, particularly at low flow rates, due to the necessarily large length-to-diameter ratio of the capillary flow channel.

As a solution to these limitations, a high flow rate (HFR) SFC-MS interface was recently developed with the aims of: a) operating at high fluid flow rates, b) improving control of restrictor temperature, c) enhancing sensitivity for less volatile compounds, and d) allowing more rapid restrictor replacement and operational convenience. The HFR interface for SFC-MS is illustrated in Figure 1. The interface has advantages (compared with earlier SFC-MS interfaces) of allowing operation at higher flow rates, providing more efficient analyte transfer to the CI region, unrestricted CI operation at optimum pressures, and maintenance of normal CI reagent gas flow rates. Although the actual sample size typically injected on column is much greater than for conventional capillary SFC, sensitivity in terms of sample concentration is enhanced, and the actual amount of sample utilized is effectively unchanged (because of the usual waste involved in loading the high pressure injection valves unless extraordinary measures are taken). The improved sensitivity was most obvious for higher molecular weight components, which we attribute to an enrichment process due to alignment of the capillary restrictor with the CI sample entrance orifice (see Figure 1).

An example of a separation for a mixture of organophosphorous pesticides obtained using the HFR SFC-MS interface is shown in Figure 2. The total ion chromatogram shows that most components are separated using a short 10-cm "microbore" column (1-mm-i.d., 5- μ m carbon-18 particle packing) with a

carbon dioxide-2% isopropanol fluid mixture as the mobile phase. The single ion chromatograms in Figure 3 indicate that detection limits of less than 10 ppb are readily obtained.

More polar and high molecular weight compounds strain the solvating power capabilities of lower temperature supercritical fluids; however, some surprisingly "difficult" compounds can be addressed. As an example, Figure 3 shows the separation and mass spectrum of cyclosporin A (MW = 1202 dalton) obtained using a carbon dioxide-2% methanol mobile phase with a microbore column having a C₁₈ stationary phase. The relatively poor separation is due to sample adsorption and is typical for polar materials which approach the limit of specific fuel consumption (SFC) applicability. The separation of more polar analytes would require more polar supercritical fluids (e.g., NH₃), but such fluids present difficulties due to the chemical stability of available stationary phases, the fused silica capillary tubing, and other materials used in SFC systems (e.g., polymeric materials such as polyimide in valves and unions). The rapid nucleation of the analyte during expansion in the interface can also be a limitation. It also appears that the specific chemical interactions in supercritical fluids (i.e., hydrogen bonding) are generally reduced compared to the liquid phase. Highly polar solvents, such as water, are often impractical because of high critical temperatures, and useful fluid mixtures are defined (and limited) by their phase equilibria.

As a practical matter, the current applicability of SFC-MS with less polar mobile phases (e.g., CO₂, propane, etc.) can be estimated on the basis of liquid solvents. Supercritical fluid chromatography appears applicable to materials soluble to any significant degree in hexane and to a large portion of compounds soluble in methylene chloride. On the other hand, SFC with less polar supercritical fluid mobile phases is unlikely to be successful if an aqueous solvent is required. Compounds that must exist as ions in solution are usually excluded. A potential method for extending SFC to highly polar and ionic compounds using reverse micelle supercritical fluid mobile phases has recently been developed at PNL, but extension to SFC-MS remains uncertain. New methods are currently under development aimed at the further expansion of SFC-MS applicability.

FIGURE 1. Schematic Illustration of the High Flow Rate Interface Allowing Improved Packed Column and Capillary SFC-MS

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FIGURE 2. Total Ion Chromatogram and Single Ion Chromatograms for an Eight Component Mixture of Labile Organophosphorus Compounds Separated Using the HFR SFC-MS with Fluid Density. a) chlorpyrifos methyl; b) chlorpyrifos; c) iodofenphos; d) leptophos; e) methidathion; f) tetrachlorvinphos; g) phosmet; h) famphur.

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FIGURE 3. SFC-MS Peak and Spectrum for Cyclosporin A

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DEVELOPMENT OF CAPILLARY ZONE ELECTROPHORESIS-MASS SPECTROMETRY

H. R. Udseth and R. D. Smith

The chemical analysis of complex mixtures continues to pose tremendous challenges. Gas chromatography-mass spectrometry (GC-MS) has become established as the definitive technique for the analysis of many compound mixtures due to its high selectivity and excellent sensitivity. The two independent dimensions of information provided by the high resolution separations of capillary GC and the mass spectra permit rapid detection and identification of most amenable compounds with an extremely high degree of confidence. Unfortunately, only a small fraction of the compounds of possible interest to many areas of research or having practical importance can be addressed by GC-MS. This limitation results from the necessity of a minimal vapor pressure and thermal stability of the analyte in both the chromatographic separation and transport through the mass spectrometer interface (where gas phase electron impact or chemical ionization methods are used almost exclusively).

Capillary zone electrophoresis (CZE) is a form of free zone electrophoresis conducted in small diameter capillaries and capable of ultra-high resolution separations. The CZE instrumentation is easily assembled and separations obtaining more than 10^6 theoretical plates in less than twenty minutes have been demonstrated. Separations are based on differences in the electrophoretic mobilities of analytes. Electro-osmotic flow of the buffer medium makes it an elution technique which resembles chromatography. Thus, CZE provides high resolution separations and selectivities which can be manipulated by changing the electrophoretic medium (i.e., typically pH and buffer composition as well as equilibria with migrating micellar phases). Capillary zone electrophoresis is amenable to broad compound classes with application limited only by the necessity for solubility in the buffer and a non-zero net electrophoretic mobility. This technique is limited to small capillary diameters (generally $<100\text{-}\mu\text{m}$ i.d.) to suppress convection caused by a radial temperature

gradient through the electrophoretic medium from Joule heating. This allows the high efficiencies resulting from the nearly flat flow profile provided by electro-osmosis to be realized. The major limitations of CZE are related to the sensitivity and range of application possible with on-column detection methods [ultraviolet (UV) and fluorescence].

Recently we described the first on-line combination of CZE with mass spectrometry, which also represented the first reported direct combination of any electrophoretic separation technique with mass spectrometry (Olivares et al. 1987). This development was based upon the recognition that both ends of the CZE capillary did not have to be immersed in buffer reservoirs, as conventionally practiced. This provided a basis for new detection methods in which the electro-osmotically induced flow could be analyzed at the column terminus. The strong electro-osmotic flow in CZE, which results from the strong zeta potential of most amenable capillary surfaces, is sufficiently large under many conditions to result in elution of ions having both positive and negative electrophoretic mobilities in a single separation. Nonaqueous buffers also allow compounds to be separated which are somewhat less polar than feasible in aqueous systems, effectively providing a range of applications which should overlap with those of SFC.

The CZE-MS combination represents a new instrumental method which has already demonstrated exceptional promise (Olivares et al. 1987). The instrumentation developed at Pacific Northwest Laboratory (PNL) is schematically illustrated in Figure 1. The interface is based upon an electrospray ionization (ESI) source which allows ion production at atmospheric pressure from an electrically induced nebulization process at the end of the CZE capillary. The highly charged droplets are exposed to a counter-current flow of dry nitrogen, which facilitates evaporation and causes the droplets to reach their maximum charge. Subsequent evaporation results in droplet breakup, producing smaller droplets which proceed along a similar course, or direct field-assisted ion desorption (or evaporation) from the droplet surface. The precise sequence of events, the competition between the above processes and their dependences upon droplet size, as well as the role of other possible contributions, remain uncertain. However, the net result of the ESI process is the production of analyte ions in a gentle process not requiring heat (other

FIGURE 1. CZE-MS Instrument Schematic

than the flow of dry nitrogen, which can be heated to 60 to 80°C). The 3- to 6-kV potential difference used in ESI can produce positive or negative ions, depending on field polarity. In our current instrumental configuration, we have utilized a three-stage vacuum system incorporating cryogenic second stage pumping and an RF quadrupole lens which transmits ions efficiently to the analysis quadrupole. The mass spectrometer is similar to systems developed commercially for atmospheric pressure ionization and such instruments can be interfaced for CZE-MS.

Because CZE separations are based on analyte charge state in solution and the ESI process appears to function most effectively for ionic species, the CZE-MS combination should be quite complementary. The ESI spectra are often dominated by molecular ions (i.e., protonated or cation adducts for the positive ion mode of operation), and multiply charged species and clusters can become significant under some conditions. Figure 2 gives an ESI spectrum for vasotocin, a polypeptide of molecular weight 1050, which shows abundant singly and doubly charged molecular ions, as well as other contributions of multiply charged species and clusters. The mass spectra are sensitive to ESI conditions, and quite different spectra can be obtained at different voltages or with other solvent systems. Our results suggest that the multiply charged spectra obtained under some conditions may be useful for compound identification.

Figure 3 shows a CZE-MS total ion electropherogram obtained for a mixture of quaternary ammonium salts for the conditions noted. The number of theoretical plates in the separation ranges from 160,000 for the trimethylphenylammonium ions to 330,000 plates for the last peak (tetrabutylammonium ions), an order of magnitude better than obtainable by liquid chromatography in similar time. In this case, sample sizes were ~300 femtomoles; however, detection limits of ~10 attomoles are obtainable using single ion detection. Thus, the CZE-MS approach offers previously unobtainable separation efficiencies (for the combination with mass spectrometry) as well as detection limits which can greatly surpass existing methods. Of course, the excellent detection limits are a necessity due to the small sample volumes injected for CZE (<100 nL). However, this combination of characteristics makes CZE-MS uniquely suited for application to biochemical samples. We anticipate that substantial gains in separation quality and detection limits will occur as our understanding increases of both CZE and the electrospray ionization interface.

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FIGURE 2. Electrospray Ionization Spectrum of Vacotocin

FIGURE 3. Total Ion Electropherogram from a CZE-MS Separation of Quaternary Ammonium Salt Mixture

• Lasers in Analytical Chemistry

Lasers have unique properties that may be used to improve both sensitivity and selectivity in analytical measurements. Pacific Northwest Laboratory is developing a number of laser-based methods for the measurement and identification of trace levels of radionuclides and environmentally hazardous materials. Over the last year we have improved the techniques of continuous-wave double-resonance ionization mass spectrometry, increasing both sensitivity and selectivity in the spectroscopic process to the point where it may be considered a near ideal atom counter, providing ionization efficiencies approaching unity and isotopic abundance sensitivities in excess of 10^{10} . With completion of the excitation process development, we have shifted emphasis in the program from spectroscopic studies to the development of methodologies for the analysis of small discrete samples. A miniature graphite furnace has been incorporated into the system for the atomization of solid and aqueous samples. Using this furnace, instrumental detection limits of 10^{-17} grams have been demonstrated for rare isotopes of barium.

ULTRA-TRACE ISOTOPIC ANALYSIS BY DOUBLE-RESONANCE IONIZATION SPECTROSCOPY

B. A. Bushaw and G. K. Gerke

Ionization spectroscopy with high-resolution, continuous-wave (cw) lasers is attractive for detecting ultra-trace quantities of rare isotopes because of the selectivity, high efficiency, and low background that can be achieved. In previous work (Cannon, Bushaw, and Whitaker 1985), we have shown isotopic selectivity in a double-resonance ionization process employing two high-resolution dye lasers and a cw Nd:YAG laser. We have recently improved both selectivity and sensitivity in the process by exciting to higher-energy Rydberg states and replacing the Nd:YAG laser with a cw CO₂ laser (Bushaw et al. 1987; Whitaker et al. 1987). During the past year we have investigated autoionizing resonances to improve ionization efficiency and reduce laser power requirements. Also, a miniature vacuum-compatible graphite tube furnace has been designed, constructed, and tested for the atomization of discrete samples.

Observed improvements in selectivity (several orders of magnitude) can be attributed to the long lifetimes of high-lying Rydberg states that are populated in the double-resonance excitation process. These lifetimes (several hundred nanoseconds) are typically a factor of 10 greater than those of the previously studied intermediate states, resulting in a hundred-fold reduction in the tails of the Lorentzian component of the lineshape. Figure 1a shows a typical total ion spectrum obtained by scanning the second dye laser over the $6s16d\ 16^1D_2$ Rydberg state in barium. Peaks due to the 0.1% abundant 130 and 132 isotopes are observable even though they are shifted only

68 and 40 MHz from the predominant (71%) 138 peak. Figure 1b shows the mass spectrum of the ions produced with both dye lasers tuned to the double resonance condition for the 130 isotope. Ninety-eight percent of the ions produced are from this 0.1% abundant isotope, indicating an abundance sensitivity of 5×10^4 against the sum of all other isotopes. Coupling the selectivity of the laser excitation with the mass discrimination of the quadrupole filter provides an overall abundance sensitivity of $\sim 10^{10}$.

Use of a CO₂ laser to ionize Rydberg-state atoms has improved sensitivity by approximately two orders of magnitude. All steps in the photoionization process can now be saturated and ionization efficiencies of up to 30% have been achieved. The improvement may be attributed to three factors: 1) longer-lived Rydberg states allow longer "access" time for photoionization, 2) the photon flux of the CO₂ laser is 10 times greater per watt than that of the previously used cw YAG laser, and 3) the density of Rydberg states and line tunability of the CO₂ laser increases the probability of accessing autoionizing resonances. With the proper choice of Rydberg state and CO₂ laser line, photoionization may be saturated at intensities of less than 100 W/cm². Figure 2a shows two spectra obtained by scanning the CO₂ laser line while the dye lasers are set for excitation to the $n = 16$ and $19\ 1D_2$ Rydberg states. While no autoionizing resonances are observed from the $n = 19$ state, two are observed from $n = 16$. The first, marked P, corresponds to a known resonance observed in absorption spectroscopy from the ground state, while the second, marked F, is a newly observed state. Figure 2b shows saturation of photoionization with increasing CO₂ laser intensity at the P resonance.

FIGURE 1. a) Isotope Shifts in the $6s16d\ ^1D_2$ State of Barium. b) Mass Spectrum of Ions Produced with Lasers Tuned for Excitation of the Mass 130 Peak in A.

FIGURE 2. a) CO_2 Laser Scans from Rydberg States Which Do and Do Not Exhibit Autoionizing Resonances. b) Saturation of Photoionization.

Measurements on discrete samples atomized in a graphite furnace have shown sensitivities of better than 0.5 attograms of sample to obtain one ion count. This corresponds to one count for every 2000 atoms placed in the furnace. This efficiency is limited primarily by conversion of salts to the neutral atomic form (atomization) within the

oven (~10%) and transport of atoms from the furnace to the ionization region (~4%). Analysis of background count rate and counting statistics yields an instrumental detection limit (3σ) of 8 attograms or 3×10^4 atoms. Actual analytical detection limits are determined by contamination of the graphite furnace material with the analyte

material. In our studies on barium, this background has been in the femtogram-to-picogram range, depending on the isotope of interest. We expect that analytical detection limits for very rare isotopes, where contamination is not a problem, will approach the instrumental detection limits and lie in the low attogram range.

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Physical Processes
in Radiation
Biology

• Radiation Physics

Initial Interaction Processes

The initial spatial distribution of energy deposition by ionizing radiation plays a significant role in the subsequent evolution of biological damage. For radiation of high linear energy transfer (LET), radiochemical and radiobiological models of radiation action rely heavily on an accurate description of the structure of charged particle tracks. Our studies concentrate on the measurement of interaction cross sections representative of charged particle interactions with constituents of tissue and the incorporation of these data, and that from the literature, into comprehensive Monte Carlo descriptions of particle tracks. Experimental studies emphasize measurements of ionization cross sections, including the energy and angle of emitted electrons, as well as the final state of the target atom or molecule. Track structure models focus on incorporation of a comprehensive data base and calculation of energy deposition spectra in microscopic sites of subcellular or molecular dimensions.

During the past year our cross-section studies here focused on interpretation of data for multiple ionization of atomic targets by light ions. Analysis of doubly differential cross sections for electron ejection from water vapor by oxygen ions has continued, with emphasis on ion charge state effects. A theoretical study of ionization of water vapor by electrons was also completed. Monte Carlo modeling has focused on determination of systematics for ionization distributions for a wide range of site sizes and in application of track structure codes to interpretation of energy transport mechanisms in deoxyribonucleic acid (DNA).

MULTIPLE IONIZATION OF ATOMS BY ION IMPACT

R. D. DuBois

In order to model the energy deposition of fast ions as they slow down in gaseous media, information about the ionization occurring in collisions between ions and target atoms/molecules is required. Our measurements of doubly differential electron emission cross sections provide detailed information about the ionization process but do not provide any information about the final states of the target. They also do not distinguish between the emission of one or more target electrons in a single collision. It is important to know the relative importance of multiple-, with respect to single-, target ionization in order to accurately model the energy deposition. In addition, multiple ionization of the media can have significant ramifications in understanding radiation-induced damage. For example, in the case of multiple-target ionization, several electrons can be ejected from a single collision site. In a proper model, each of these electrons and the subsequent ionization that is produced must then be accounted for. Also, the multiply charged positive ion produced at the collision site can interact with its neighbors, but it will interact differently than would a singly charged ion. Thus, the importance of

multiple ionization with regard to radiation damage is that it may produce "hot spots" along the projectile track. Furthermore, the multiply charged target ions produced at these hot spots can lead to chemical effects that are entirely different from those involving only singly charged ions.

Thus, in order to investigate the relative importance and details of multiple ionization, we have for the past several years experimentally studied multiple ionization occurring in ion-atom collisions. Atomic targets were investigated since the data are considerably easier to accumulate and to interpret. In addition, considerable ionization data are available for atomic targets. In our studies, absolute cross sections for single- and multiple-target ionization were measured for collisions between various projectile ions and atoms. The range of impact energy studied was from 15 to 2000 keV. From these data, we also obtained absolute cross sections for the total positive charge production, for the free electron production, and for charge transfer. Thus, from a single measurement, a significant amount of information was obtained.

To date, multiple ionization of He, Ne, Ar, Kr, and Xe targets has been studied. Primarily, H and He ions were used, although some data for heavier ions (C, N and O) have

also been obtained. Generally, lower projectile charge states were used, but neutral particle impact has also been briefly investigated. Cross sections for multiple-target ionization resulting from direct ionization as well as from charge-transferring collisions were measured. From the data it was often possible to determine whether the multiple ionization was the result of outer- or inner-shell ionization.

Because of the large amount of data that has been collected over several years, only a summary of our results and interpretations will be given. The following aspects of multiple target ionization have been experimentally investigated and from the accumulated data, the following generalizations can be made.

a) The relative importance of multiple-target ionization occurring in light ion-atom collisions was measured (DuBois, Toburen, and Rudd 1984; DuBois 1986, 1987a). The percentage of multiple ionization with respect to the total ionization can be large (>50%) or small (<<1%), depending on the impact energy and on the collision partners. It tends to be larger for the heavier targets (Ar, Kr, Xe) than for the lighter targets (He, Ne). Also, for any specific target, the amount of multiple ionization tends to increase as the projectile becomes heavier and particularly as the projectile charge increases.

b) The relative importance of directly ionizing and charge transferring collisions was investigated (DuBois 1984, 1986, 1987a). The total multiple ionization is the result of directly ionizing and charge transferring collisions. At lower impact energies (when the projectile velocity is comparable to, or less than, the bound electron velocity) electron capture by the projectile plus simultaneous ionization of additional target electrons is the dominant contributor to the total multiple ionization cross section. Note that this mechanism was shown to be the dominant free electron production mechanism in certain cases. At high impact energies, direct multiple target ionization dominates. Also at high impact energies, electron loss by the projectile can lead to high probabilities for simultaneous projectile/multiple-target ionization. This can be quite important since the modeling of these collisions must now account for the ionized projectile and its free electron as well as the multiply ionized target and its free electrons.

c) The contribution of outer- and inner-shell ionization to multiple ionization was

determined for several atomic targets (DuBois and Manson 1987). Multiple-target ionization, occurring either via directly ionizing or charge-transferring collisions, results from either multiple outer-shell ionization or from inner-shell ionization followed by Auger or x-ray relaxation processes. In the case of proton impact, the multiple outer-shell ionization processes were shown to dominate for the lighter targets (Ne) or for projectile velocities smaller than the inner-shell bound electron velocities. For the heavier targets (Ar, Kr), inner-shell ionization is totally responsible for the observed multiple ionization at higher impact energies.

d) The impact mechanism leading to direct multiple outer-shell ionization was determined (DuBois and Manson 1987). The removal of several electrons from an atom in a single collision can occur by a single projectile-target interaction where one electron is ejected. This electron can then interact with other electrons, causing them to be ejected. On the other hand, the projectile can be thought of as interacting with each electron independently such that each electron is ejected. Using our available data for proton impact on atoms, it was shown that the direct multiple outer-shell ionization can be well represented over a broad energy range by the multi-interaction picture. In this picture, a simple independent electron model can be used to determine the cross sections for ionizing q electrons from a shell containing n electrons, i.e.,

$$\sigma_q = 2\pi \int_0^\infty \binom{n}{q} P(b)^q [1-P(b)]^{n-q} b db$$

As a reasonable, but simple, approximation, the ionization probability is assumed to decrease exponentially as a function of impact parameter (i.e., $P(b) = P(0)e^{-b/R}$, where R is a characteristic distance roughly corresponding to the shell diameter). Thus, direct multiple-ionization cross sections can be easily calculated provided that $P(0)$ is known. $P(0)$ can be determined from the experimental direct single-ionization cross section by using the above formula.

e) The scaling of direct multiple-ionization cross sections was investigated for various projectile ions (DuBois 1987b). Data for direct single and double ionization of helium by various projectile ions were analyzed using the above independent electron formulation. The available data included projectile ions ranging from protons to uranium, charge states ranging from

0 to 44+, and relative charge states ranging from fully clothed to fully stripped. Impact velocities investigated ranged from approximately 50 to 2000 keV/amu. The analysis indicates that in this energy range, double ionization of helium is the result of a multiple projectile-target interaction. The analysis also indicates that the single- and double-ionization cross sections scale as Z^2 and Z^4 , respectively, for small values of Z/v and with a slower dependence otherwise. Here, Z is the nuclear charge and v is the impact velocity. This is valid for fully stripped ion impact. In the case of partially clothed ion impact, Z is an effective charge since the bound electron partially screens the nuclear charge. One important result of this analysis was that the effective charge is a function of the impact parameter and hence this dependence $[Z(b)]$ must be included to obtain the proper scaling. Although this analysis was done for a He target, a small amount of data are available for the Ne target. These data indicate that the same picture can be used for other atoms as well.

At this point, our studies of multiple ionization will now concentrate on molecular targets. What we have learned from atomic targets provides a good basis for understanding multiple ionization of simple molecules. Cross sections for molecular dissociation into one or more charged fragments will be measured and hopefully understood. Multiple ionization of atoms will continue for selected systems as the need arises.

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ELECTRON EMISSION FROM SOLIDS

R. D. DuBois

An apparatus for studying the doubly differential electron emission from various solid (foil) targets is being constructed. This apparatus will consist of an ultra-high-vacuum system containing a precision manipulator for foil targets. A rotatable time-of-flight electron analyzer will be used to detect electrons emitted during projectile ion-target interactions.

To date, an ultra-high-vacuum chamber and pumps have been purchased. The chamber stand and hoist for the lid have been constructed. A double-walled magnetic shield has been designed and is on order. It is anticipated that during the next year the chamber will be fully assembled and a time-of-flight electron detector will be designed, constructed and installed, at which time preliminary testing will begin.

DOUBLY DIFFERENTIAL IONIZATION CROSS SECTION FOR THE INTERMEDIATE-VELOCITY HEAVY IONS

L. H. Toburen, R. D. DuBois, and P. Goldhagen*

For a complete description of the energy deposition by fast neutrons in tissue, one must understand the interaction process for charged-particle recoils including hydrogen, helium, carbon, oxygen and nitrogen ions. Although our primary emphasis has been on hydrogen and helium ions, the predominant particles induced by neutron reactions in tissue, we have also initiated measurements for carbon and oxygen ions. During the past year, analysis of data of the doubly differential cross sections for interaction of oxygen ions with water vapor molecules has continued. The data for ion energies from 1.3 to 3.3 MeV and charge states 1 to 3 enable a detailed study of the charge state dependents on electron emission cross

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sections. Cross sections measured in FY 1986 are available for electron emission angles ranging from 15 to 130 degrees and ejected energies from a few eV to 1500 eV.

As a first step in the data analysis, a careful study of the uncertainties in the absolute cross sections was undertaken using the calibration data available. Accurate absolute values are necessary if reliable comparisons are to be made to measurements made for other ions and other target systems. Unfortunately, inconsistencies in relative yields between various data available introduce sizeable uncertainties in our present knowledge of the absolute yields. In order to reduce these uncertainties it will be necessary to make further

measurements for both oxygen ions and protons; the proton data can then be used to place the yields with greater certainty.

Although uncertainties in the absolute values of measured cross sections preclude reliable comparison of the oxygen data to cross sections obtained for other ions and targets, they still provide a self consistent set of results for the study of ion charge state dependence on emission cross sections. We have looked at the effective charge for interactions of O^{n+} , $n=1$ to 3, with water molecules and compared these results with previous studies for He^+ and He^{2+} ions. The results of this comparison are summarized in Figure 1. Two rather interesting results are illustrated. First, the

FIGURE 1. Charge State Dependence for Ionization of Water Vapor by Fast Oxygen Ions. The insert indicates the effective charge of each ion as a function of energy loss by the different ion charge states. The solid line illustrates the functional dependence obtained from a model previously used for helium ions.

dependence on energy loss (related to ejected electron energy) is quite different for oxygen ions from that previously observed for helium ions, which are represented by the solid line in Figure 1. Second, the yield of electrons ejected with very low energies differ greatly from a Z^2 dependence, where Z is the net charge on the ion. This is at odds with the assumption that at sufficiently large impact parameters, which are involved with small energy losses by the primary, the ion should appear as a point charge given by its state of ionization.

Results of this study were presented in a paper entitled "Secondary Electron Production by Heavy Charged Particles" at the Eighth International Congress of Radiation Research (Edinburgh, Scotland). They illustrate the important need for further studies of structured ions if we are to understand the interaction process. Additional data are required to place the oxygen ion results on an accurate absolute cross-section basis for comparison with data available for other ions. In addition, a continued study of charge state effects is required for other ions in order to determine cross-section systematics for model development.

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DIFFERENTIAL CROSS SECTIONS FOR IONIZATION OF WATER BY FAST ELECTRONS

J. H. Miller and S. T. Manson*

A recent publication in the *Journal of Chemical Physics* (Miller et al. 1987) compared experimental cross sections for ionization of water molecules by fast electrons (Bolorizadeh and Rudd 1986) with calculations based on a semi-empirical model. The following two exceptions to the general agreement between theory and experiment were noted: 1) experimental cross sections for production of electrons in the final state with energy roughly half of the initial electron energy, where the differential ionization cross section is smallest, were 3 to

5 times larger than calculated results; 2) the model predicted results more than an order of magnitude larger than were observed experimentally for electrons in the final state with energy nearly equal to the initial electron energy.

To increase the accuracy of experimental data for collisions with small cross section, Professor Rudd modified the experimental apparatus to better screen the detector from spurious electrons. Figure 1 compares results obtained with the modified instrument to our model predictions. The peak in the experimental data, when the energy of electrons detected after the collision is about one third of the initial electron energy, is due to the production of Auger electrons that are not included in our model of the continuum spectrum. Greater suppression of spurious electrons improved the agreement between theory and experiment near the minimum in the differential cross section, but the experimental data in this energy region are still about twice as large as results predicted by our model. The residual discrepancy between theory and experiment may be due to collisions that result in multiple ionization of the target. These processes, which are not included in our model, may not be negligible at energies where the differential cross section for single ionization is small.

The source of the difference between theory and experiment for electrons in the final state with energy near the initial energy is probably due to their angular distribution. Most of the electrons which appear in this energy range after the collision are primary electrons that have sustained only a small energy loss and angular deflection. These electrons will not be seen by the experimental apparatus, which has a minimum detection angle of 15 degrees. Consequently, the singly differential cross section, which is obtained by integrating the observed doubly differential cross section over the scattering angle, will be too small.

Since the doubly differential cross section for small energy loss and angular deflection of a fast electron can be predicted from the optical oscillator strength of the target, we have attempted to correct the observed singly differential cross section for electrons scattered into angles smaller than 15 degrees. These results, which are shown by the open squares in Figure 1, are in better agreement with the predictions of our semi-empirical model.

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FIGURE 1. Cross Section, Differential in the Energy of Electrons After the Collision, for Ionization of Water by 1.5-keV Electrons

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Track Structure

The initial spatial pattern of energy deposition by ionizing radiation plays a significant role in the subsequent evolution of biologically active chemical species. Radiochemical and radiobiological models of radiation action initiated by high linear energy transfer (LET) particles rely heavily on an accurate description of charged particle tracks. Our study of track structure includes both theoretical and experimental investigation of the spatial pattern of energy deposition. Incorporation of these data into models of energy transport in condensed phase clarifies the effects of the initial spatial patterns on evolving chemical yields. Because of the experimental limitations on determination of energy deposition in nanometer-sized volumes, we have continued to develop Monte Carlo codes to calculate ionization and energy imparted in small sites. During the past year, we extended our study of the systematics of energy deposition in small sites to include energy deposition by ions just outside the volume of interest and completed our characterization of those events produced by ions that cross the site. The Monte Carlo codes were also extended for calculation of energy deposition by heavy (HZE) particles to begin to provide stochastic information of interest to the radiation biology of high-energy heavy ions. The modeling of stochastic processes in the transfer of energy in macromolecular systems focused on understanding anisotropic radical yields observed in neutron irradiation of oriented deoxyribonucleic acid (DNA) fibers. These studies have benefited from close collaboration with other researchers around the world, including H. G. Paretzke, GSF-Institut für Strahlenschutz, Neuherberg, West Germany; D. E. Charlton, Concordia University, Canada; J. J. Coyne, National Bureau of Standards (NBS); L. S. Myers and C. Swenberg, Armed Forces Radiobiology Research Institute (AFRRI), Bethesda, Maryland; T. L. Criswell, Boeing Aerospace Company, Seattle, Washington; and D. Goodhead, Medical Research Council (MRC), Harwell, England.

IONIZATION IN SMALL SITES

W. E. Wilson, N. F. Metting, and
H. G. Paretzke*

Our treatment of the ionization and energy deposition in submicron sites produced by crossing protons was significantly reworked. A theoretical basis for the major part of the dependence on ion energy and site size was developed. Also, an analytical solution to an equation was discovered which obviated a requirement for a numerical solution. These improvements resulted in a more sound and concise algorithm for describing the distributions.

Two papers on the results were prepared; one was submitted to Radiation Research Journal, the other paper was presented at the Sixth Symposium on Neutron Dosimetry (October 12-16, 1987, Neuherberg, Federal Republic of Germany).

The two abstracts are presented here.

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MICRODOSIMETRIC ASPECTS OF 0.3- TO 20-MeV PROTON TRACKS, I. CROSSERS^(a)

W. E. Wilson, N. F. Metting, and
H. G. Paretzke*

Track structures of fast protons obtained by computer simulation using a detailed-history Monte Carlo method were evaluated to obtain stochastic frequency distributions for energy imparted and number of ionizations. These microdosimetric quantities were calculated for 0.3- to 20-MeV protons passing through spherical sites of 2 nm to 1 μ m diameter in unit density water. From the distributions, the dependencies of their first two moments on ion energy, site size, and position of the track within the site were computed. The frequency distributions were fitted by simple analytical functions to

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fitted by simple analytical functions to facilitate their use in further microdosimetric considerations.

AN ANALYTIC MODEL FOR IONIZATION DISTRIBUTIONS PRODUCED IN NANOMETER VOLUMES BY RECOIL PROTONS^(a)

W. E. Wilson and H. G. Paretzke*

A concise mathematical model is presented for the microdosimetric ionization distributions produced in subcellular volumes by energetic protons of 0.3 to 20 MeV. The model is based on the results of extensive Monte Carlo computer simulations of high-LET charged particle tracks and can be used to estimate, with only a modest computing effort, straggling and delta-ray transport effects in the nanometer dosimetry of high-LET radiations.

LOW-ENERGY ELECTRON MICRODOSIMETRY

W. E. Wilson, with J. H. Miller and T. Straume**

For the purpose of evaluating the dosimetry of continuum photon irradiation of oocytes, we have developed a low-energy (secondary) electron transport (Monte Carlo) code by adapting portions of MOCA14. The code allows an isotropic but otherwise arbitrary source spectrum up to 100 keV. Between primary energies of 10 and 100 keV, a straight-ahead approximation is assumed. The simulated oocyte target components are a spherical nucleus of 5 μm diameter concentric with a 6- μm -diameter cell enclosed by a 100-nm-thick membrane. This target is irradiated isotropically by a continuum spectrum calculated (by LLNL) for a 250 kVp x-ray source. For reasons of computer economy the source domain is restricted to less than the maximum range of the most energetic electron. Domains of 20 and 40 μm are being

compared for variance from electronic equilibrium. Correlated single-event distributions are obtained for simultaneous energy deposition in the nucleus and in the surrounding cell membrane. Preliminary results indicate that a significant fraction of the single events in the nucleus are accompanied by little or no energy deposition in the membrane.

MICRODOSIMETRIC ASPECTS OF 0.3- TO 20-MeV PROTON TRACKS, II. TOUCHERS

W. E. Wilson and H. G. Paretzke*

Protons that pass outside the site but which deposit energy via secondary electrons, so-called "touchers," are significant events for very small sites inasmuch as nearly 50% of the stopping power is borne by delta-rays. Toucher-event modeling has focused on trying to discover the systematics of the ionization distributions. We reported last year (Wilson and Paretzke 1987) that the probability for a nanometer site experiencing any ionization deposition was proportional to the solid angle of the site as seen from the ion path, and to the total ionization cross section, i.e., proportional to the number of delta-rays produced per unit path length.

This year we scaled the data by the real trigonometric solid angle for near distances, tried various effective eccentricities, and used partial cross sections that realistically reflect the production of only those delta-rays capable of reaching the site. Some of the variations were helpful over small regions of the parameters, ion energy for example. But none, alone or collectively, produced universal simplicity or insight into the problem. We did conclude that our set of computed ionization distributions is inadequate for the task. Additional computed cases are on order for next year.

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MODELING FREE RADICAL YIELDS IN DNA EXPOSED TO HIGH-LET RADIATION

J. H. Miller, W. E. Wilson, and
C. E. Swenberg*

Experiments performed at the Armed Forces Radiobiology Research Institute (AFRRI) with samples of oriented DNA exposed to neutrons suggest that free-radical yields depend upon the orientation of DNA molecules relative to the recoil proton flux (Arroyo et al. 1986). We have developed a model for this effect based on Monte Carlo simulation (Wilson and Paretzke 1981) of the pattern of energy deposition in DNA by protons slowing down in the sample and assuming more rapid migration of energy along DNA chains than between DNA molecules.

The results of our stochastic calculations are summarized in Figure 1. The enhancement factor is the ratio of the yield of 5,6-dihydro-5-thymyl (TH) radicals when the proton flux is parallel to the helical axis of

DNA to the yield when the flux is perpendicular to the molecular orientation. The abscissa is the width of a Gaussian distribution function that approximates the spatial delocation of energy in collective modes of excitations (plasmons) that are intermediates in the absorption of ionizing radiation. The width is expressed in units of the stacking distance between adjacent base pairs, which is about 3.4 Å in the B conformation.

The increase in the enhancement factor with increasing initial width of the plasmon state is a consequence of the fact that the yield of TH is mainly determined by the activation energy for proton transfer to the thymine radical anion (T^-). As energy from a given energy deposition event in DNA becomes more diffuse, its effectiveness in activating proton transfer to T^- is reduced. This has a greater impact on the yield of TH in the perpendicular than in the parallel case because the pattern of energy deposition in the former is characterized by small isolated clusters of ionizations. Only in the parallel irradiation geometry is there a significant probability of a large number of energy deposition events occurring in the

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FIGURE 1. Enhancement of the Yield of 5,6-dihydro-5-thymyl Radicals in DNA That Is Oriented Parallel to a Flux of Proton Irradiation. Initial width refers to the spatial delocation at early times of energy deposition events in the molecule. Solid symbols show the effect of a ± 10 -degree uncertainty in the orientation of DNA molecules relative to the proton flux.

same molecule. Intramolecular energy transfer in DNA allows multiple energy deposition events in the same DNA molecule to contribute collectively to activation of proton transfer to T⁻. In this collective mode, the effectiveness of individual energy deposition events is less important.

Hence, our interest in the unusual orientation dependence of radical yields results mainly from its implications for energy transfer, both on the very short time scale of production and decay of plasmon states and the longer time scale characteristic of energy migration in DNA. If our proposed mechanism for the orientation effect is correct, then its magnitude at one proton energy will give an indication of the value of the initial width parameter. Under the reasonable assumption that delocalization of

plasmon states is independent of the linear-energy-transfer of the radiation, we can use this value to predict the magnitude of the orientation effect at other proton energies. The correctness of these predictions will be a crucial test of the assumptions of our model.

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• Radiation Dosimetry

Dosimetry

The primary goal underlying the Radiation Dosimetry task is to understand the connections between the physical events produced by the interaction of ionizing radiation with matter and their biological consequences. Dosimetry concentrates on the physical aspects of the problem, utilizing fundamental physical data to describe the interactions and organizing the results in ways that can be correlated with the biological effects. Special mathematical and experimental techniques are being developed to explore the differences in the energy deposition characteristics of various radiations in an effort to understand the effects of these radiations in radiobiology research and in the environment. Experimental tests of specific aspects of track structure calculations are conducted to test the completeness of these models and the adequacy of the input data. Comparisons with other methods of characterizing the energy deposition by charged particles such as the homogenous track models were also carried out. In addition, this program develops specialized dosimeters needed for the execution of these experimental tests of track structure and for the proper dosimetry of special radiation beams used in biophysical studies.

MICRODOSIMETRY OF FAST HEAVY ION BEAMS

N. F. Metting, L. A. Braby, L. H. Toburen, G. Kraft*, M. Scholz*, F. Kraske*, H. Schmidt-Böcking**, R. Dörner**, and R. Seip**

Biophysical and biochemical studies of the effects of high-LET radiation under way at numerous laboratories indicate the importance of understanding the pattern of energy deposition in volumes comparable to that of subcellular structures. The inadequacy of LET as a quantitative description of radiation quality has been shown in numerous experiments employing heavy charged particles. In order to better understand the molecular mechanisms responsible for the biological effects of high-LET radiation, it is important to obtain more detailed information regarding the stochastic nature of energy deposition in volumes of subcellular dimensions. During the past year we have completed a study of the distributions of ionization as a function of the radial distance from high-energy iron ions in collaboration with scientists from Lawrence Berkeley Laboratory (LBL) and Columbia University (Metting et al. 1987) and initiated new studies at the UNILAC accelerator at GSI-Darmstadt, West Germany.

The UNILAC accelerator at Darmstadt provides a unique opportunity to study very high-LET

ions where effects of densely ionizing tracks are emphasized. In particular, biological and biochemical response for ions of the same LET, but different species and energies, are grossly different, emphasizing the inadequacy of LET as a radiation parameter for predicting biological effects. The UNILAC accelerator is capable of accelerating ions, through uranium, to energies ranging from 1.4 to 20 MeV/amu. For uranium ions at 6 MeV/amu the LET (16 MeV/ μ m) is nearly 2 orders of magnitude greater than high energy ions from the Berkeley BEVALAC. Stochastic models of the track structure of UNILAC ions are difficult to develop because ions at these energies are not usually fully stripped of their bound electrons. At the present time little data exist, and there is no reliable theory for the spatial distribution of ionization by ions that are not fully stripped.

To gain more detailed information on the structure of high-LET charged particle tracks a collaborative effort among PNL, GSI, and the University of Frankfurt was initiated to measure the radial distribution of ionization around UNILAC ions. Using knowledge gained from similar measurements conducted at PNL (Glass and Roesch 1972; Weigand 1983) and LBL (Metting et al. 1986, 1987; Metting 1987) a new wall-less proportional counter and vacuum chamber was designed and built at PNL and shipped to GSI for these measurements. A schematic drawing of the apparatus is shown in Figure 1. The chamber, a cylinder 72 cm in diameter by 72 cm long, incorporated an entrance collimation system for precisely locating the incident beam and a proportional counter mounting system capable of two-dimensional

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FIGURE 1. Schematic of Proportional Counter and Vacuum Chamber

motion. The entrance collimation system contains several aperture sizes which can be remotely changed to accurately locate the beam and provide control of the ion fluence. The proportional counter mounting system provides remote transverse and longitudinal positioning of the wall-less proportional counter with respect to the beam. Longitudinal motion is required for testing secondary charged particle equilibrium. For these measurements a newly designed proportional counter was used, employing fine wires to define a cylindrical volume approximately 0.2 inches in diameter. (See the report immediately following for details on the detector design.)

Distributions of energy deposition were measured as a function of radial distance from the ion's path for 5.9 MeV/amu uranium, and 13.0, 13.8, 16.2 and 17.2 MeV/amu germanium ions. For these very high-LET ions,

rapid accumulation of radiation damage precludes the use of solid-state detectors for ion detection. Thus, it was necessary to use a gas-filled detector to monitor the position and quality of the primary ions. For this purpose a gas avalanche detector provided by Professor Horst Schmidt-Böcking's group at the University of Frankfurt was used. There are several anode configurations available for this detector, ranging from one-dimensional position determination to 360-degree radial position sensitivity. For this measurement we used the two-dimensional "backgammon" anode (Seip 1987). In this configuration we were able to measure the spatial x-y coordinates of each primary ion. A two-dimensional plot of a representative 5.9 MeV/amu uranium beam profile is shown in Figure 2. Data were collected via CAMAC electronics using GSI generated software (GOLDA data acquisition system)

FIGURE 2. Charged Particle Beam Profile Measured Using the Position-Sensitive Avalanche Detector. These data are for a 5.9-MeV uranium beam passing through the vacuum tank filled to 40 Torr of propane gas: a) three-dimensional profile; b) scatter plot; c) contour plot of beam intensity—contours incorporate 20%, 40%, 60%, and 80% of the beam.

into a PDP11-45 and directly onto magnetic tape. On-line data analysis and monitoring was useful for checking detector performance and for making experimental protocol decisions. Our experiment was given the status of "parasite user," and as such we were given every tenth pulse of ions produced by the accelerator. The type of ion, its energy, and its charge state were chosen by the "main user." The amount of ions available for our type of experiment was still far in excess of what was needed, but the intensity was easily and reliably controlled to our specifications by the accelerator operators.

On-line and preliminary analysis of the measured ionization distributions indicate

that the system performed well. Figure 3 shows the pulse height spectra obtained for a 0.25- μ m simulated site diameter produced by the α -particle calibration source (^{241}Am) and a 5.9-MeV/nucleon uranium ion passing one diameter from the proportional counter axis. An ion beam passing through the center of the proportional counter deposits nearly 50 times more energy than the example shown at 1 diameter from the center. We are presently analyzing the data using software specific to microdosimetry to provide detailed information on the ionization distributions as a function of ion species (U, Ge), ion energy, and radial distance. The germanium ion data will be particularly useful for testing models of the spectrum

FIGURE 3. Distribution of Ionization Events from ^{241}Am α -Particles Averaged over Chordlength Distribution (source large relative to detector) Compared to Distribution Resulting from Passage of 5.9 MeV/amu Uranium Ion One Diameter from Detector Axis. Simulated site size for these measurements was $0.25\text{ }\mu\text{m}$ in diameter.

of energy deposition at different ion velocities.

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MICRODOSIMETRY DETECTOR DEVELOPMENT

L. A. Braby and N. F. Metting

A very small detector is needed for heavy ion radial distance microdosimetry since a

small detector makes it possible to simulate somewhat smaller site sizes while maintaining secondary charged particle equilibrium in a vacuum chamber of reasonable size. The smaller actual size of the detector also allows it to measure energy deposition in volumes situated over a wider range of distances from the path of the primary heavy ion than was possible before.

The other type of microdosimetric measurement in which a smaller configuration is important is that of accelerator-produced neutrons. Because the energy and fluence of these neutrons are a function, in part, of the solid angle subtended by whatever is being irradiated (see neutron irradiation report, this section), it is best to use as small a detector as possible.

A new detector has been designed and one prototype constructed (see Figure 1). The cylindrical sensitive volume, 5 mm high by 5 mm diameter, is defined in length by field tubes, and in diameter by a circular grid of fine wires running parallel to the center anode wire. The surrounding grid is thus nearly 95% transparent to incoming radiation. The proportional counter (PC) is supported in a rigid aluminum structure which is held some distance away to minimize particle scatter into the detector. The preamplifier circuitry for this detector was also of a new design featuring a lower noise profile and a more compact form which makes it possible to mount it at the PC base, thus minimizing input capacitance and further reducing the noise. This prototype detector was successfully tested, and then used to measure ionization distributions for fast heavy ion beams at GSI-Darmstadt, West Germany (see microdosimetry report, this section). Another PC of the same design is being fabricated for use at PNL's accelerator facility.

IRRADIATION OF BIOLOGICAL SYSTEMS BY MONOENERGETIC NEUTRONS

N. F. Metting, L. A. Braby, and
M. E. Middendorf

The importance of understanding the relative biological effectiveness (RBE) of neutrons, especially for low dose-rate neutron exposures, has encouraged us to re-evaluate the use of our 2-MV positive ion Van de Graaff accelerator to generate monoenergetic neutrons for use in radiobiological studies. The five commonly used neutron-producing reactions are $\text{Li}^7(p,n)\text{Be}^7$, $\text{T}(p,n)\text{He}^3$,

FIGURE 1. A Wall-Less Proportional Counter Designed for Use in Measuring Ionization Around High Energy Heavy Ion Beams

$\text{C}^{12}(d,n)\text{N}^{13}$, $\text{D}(d,n)\text{He}^3$, and $\text{T}(d,n)\text{He}^4$. For accelerator-produced neutrons, the neutron energy and fluence vary as a function of several parameters including: the reaction (target and projectile used), target thickness, the bombarding particle energy, beam current, and the angle at which the neutron is ejected. These parameters can be chosen such that the neutrons are of the desired energy and any photon dose is minimized.

The angle at which the sample is irradiated must also be balanced with its linear distance from the target in order to maximize the neutron flux density (a function of $1/r^2$) while minimizing the change in neutron energy due to the solid angle subtended by the sample. Representative dose rates and neutron energies which have been produced by the PNL Van de Graaff are summarized in Figure 1.

The neutron irradiations are done in a large open room with a low density suspended aluminum floor to minimize backscatter. For the same reason, the incubator built to hold the cell samples during irradiation is very simply constructed. It consists of a large

aluminum hoop supported on stands, with adjustable aluminum rods on which are attached the cell containers, and a surrounding polyethylene envelope with a thermostatically controlled heater-blower.

We have obtained dose response curves for both C3H mouse 10T1/2 and mouse L cell lines as a first step in developing assays of non-lethal end points. A preliminary experiment in which plateau-phase 10T1/2 cells were irradiated with 0.35 Gy of 1.4-MeV neutrons shows a transformation frequency per surviving cell of about 1.2×10^{-4} . This value is slightly lower than the value measured in the same cell line irradiated with JANUS reactor neutrons.

FIGURE 1. Experimentally Achieved Dose Equivalent Rates as a Function of Neutron Energy, Produced by the PNL Positive-Ion Van de Graaff. Notice that the quantities are also both functions of the projectile ion energy, beam current, and angle of ejection.

Modeling and Cellular Studies

The effects of ionizing radiation on living systems begin with the physical processes of energy deposition and develop through many stages of chemical reaction and biological response. The modeling effort attempts to organize the available data and theories of all of these stages into self-consistent models that can be compared and tested. Biological and biochemical concepts are expressed in terms of mathematical relationships when possible, and the consequences of these different mechanisms are deduced. Models that are characteristic of specific biochemical mechanisms or groups of mechanisms are contrasted in order to determine whether they produce different consequences at the cellular level. Often models based on quite different assumptions about mechanisms result in equivalent dose-effect relationships, but differences can be found in predicted effects of dose protraction or other conditions that favor repair. When differences that would be statistically significant at the precision of realistic experiments are found, suitable experiments are designed. In general, experiments using a well-characterized cell line are preferred, since more can be learned about specific mechanisms and their interactions by studying one system in detail.

Mathematical modeling and cellular studies complement each other. New experimental techniques in molecular biology sometimes result in discovery of new mechanisms. When these mechanisms are included in models, the resulting changes in the predicted effects of specific irradiation regimes inspire new experiments. Often the results of these experiments show deviations from the predictions which in turn suggest other mechanisms to be tested. For example, we have recently found that mechanisms such as enzyme pool depletion, concentration-dependent misrepair, and sublethal damage interaction are not adequate to account for the observed interaction of dose and repair time on the reproductive survival of plateau-phase Chinese hamster ovary (CHO) cells. Other mechanisms, such as the effect of chromosome structure on the accessibility of damage to repair, are being investigated to determine whether they play a role.

INTERPRETING SURVIVAL OBSERVATIONS USING PHENOMENOLOGICAL MODELS

J. M. Nelson, L. A. Braby, N. F. Metting, and W. C. Roesch

We have continued our experiments to test various competing biophysical models using split-dose, delayed-plating, and low-dose-rate methods. Most ionizing radiation damage appears to be repairable, but the nature and consequences of the initial unrepaired damage remain uncertain. Saturation models assume only lethal or potentially lethal damage (damage capable of killing the cell unless it is repaired). The accumulation models are based on the assumption that some fraction of the damage is not necessarily lethal or potentially lethal, but sublethal (innocuous damage which can become lethal only after interaction with additional sublethal damage).

We generally accept that the dose-rate dependence observed in the shoulder region of the mammalian cell survival curve results from modification of damage by cellular biochemical processes. The role of these repair processes at high dose rates or with radiations having higher stopping powers is not clear. However, most endpoints studied

appear to depend on both the accuracy of the biochemical processes involved and the consequences of the unprocessed damage remaining within the cells.

If only lethal and potentially lethal damage were produced, then the observed shape of the dose-effect survival curve would have to be the result of either the saturation of a repair process or some form of interaction that causes misrepair or fixation and depends on some power, greater than one, of the concentration of damage. If we assume that part of the damage is sublethal, this itself would allow for the shoulder on the survival curve. It would be the repair of this sublethal damage, however, that would be responsible for the observed dose-rate and split-dose effects. The observed increase in the relative biological effectiveness (RBE) with increasing linear energy transfer (LET), would follow from either sublethal damage interaction or from a concentration-dependent misrepair process. However, at least in its simplest form, the repair saturation concept should be independent of LET.

We can conclude, then, that if repair saturation alone is responsible for the dose-effect relationship and we still observe an

increase in RBE with increasing LET, there must be some other type of damage produced. This would be a consequence of the higher energy deposition densities. Therefore, regardless of which type of model we consider, we must assume that there are at least two different types of damage. Part of this damage must be potentially lethal, because we observe an enhancement in survival during a delay between irradiation and plating. However, if we do not assume that part of the damage is sublethal, then we must accept the idea that there are at least two different types of potentially lethal damage to account for the observed LET effects.

Two components of enzymatic repair have been identified in our starved plateau-phase CHO cells. Both have been studied extensively by split-dose, dose-rate, and delayed-plating methods. Several observations are of particular interest. First is the fact that the repair rates and fractions of damage repaired by each of the processes appears to be independent of the initial amounts of damage produced. Although the two processes may share some common steps in the biochemical sequence, there is strong evidence to indicate that they involve different types of initial damage. This supports the conclusion that both sublethal and potentially lethal damage are produced and removed in the irradiated cell.

An extensive analysis of different types of saturation models (see Calkins 1971 and Goodhead 1986) indicates that according to the predictions of these models, survival following a plating delay should always be greater than after the same exposure split into two fractions and separated by the equivalent time for repair. This is illustrated in Figures 1 and 2, where, regardless of whether we consider one or two rate constants associated with repair and regardless of the values of these constants or the fixation times, the saturation models predict greater repair of damage (higher survival) during a delayed-plating experiment than during a split-dose experiment. However, in carefully controlled delayed-plating and split-dose experiments (Figure 3) we find that survival is increased when the dose is split into two fractions separated by 24 h and plated promptly following the second dose. In these experiments, the plateau-phase cultures were initiated at the same time, from the same source cultures, and randomly selected for one procedure or another (plating delay, split dose, etc.).

FIGURE 1. Predicted Survival as a Function of Time, Assuming a Single Use (suicide) Repair Enzyme. Regardless of whether we assume fixation immediately after trypsinization (upper panel) or some inherent repair time after replating (lower panel), split-dose survival approaches but never equals that after a plating delay.

The above comparisons with the predictions of several different models suggest that models that assume only simple types of mechanisms, such as the saturation of a rapid repair process or the interaction of pairs of sublesions, are inadequate to explain the data. However, these data may be consistent with more complex models that consider combinations of both sublethal and potentially lethal damage, or models involving multiple step processes. Because these

FIGURE 2. Predictions Similar to Those Shown in Figure 1, Showing the Effect of Varying the Relative Values of the Two Rate Constants Associated with Binding (k_1) and Release (k_2) from the Damaged Molecular Substrate

more complex models involve more parameters, simple agreement with dose-effect curves or repair data does not appear to be capable of providing an adequate test of their validity. Consequently, more complex tests, such as determining dose dependence of the repair capacity, will be employed. These studies and conclusions were reported in an invited paper.^(a)

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FIGURE 3. Experimental Survival Data from Split-Dose and Delayed-Plating Experiments Using Plateau-Phase CHO Cells. Total dose and repair intervals are equivalent in both cases.

THE MODIFICATION OF DAMAGE FOLLOWING LOW DOSES

L. A. Braby, J. M. Nelson, and N. F. Metting

As discussed last year (Braby, Metting and Nelson 1987), the nature of unrepaired damage in cells can be investigated through the response to low doses of radiation. Damage which is sublethal in nature is not expressed in a survival experiment until additional damage has accumulated and there has been an interaction. Thus, at very low doses the damage-interaction mechanism is responsible for very little lethal or potentially lethal damage, and repair of the latter should essentially disappear. An alternative model suggests that potentially lethal damage is either repaired with a constant half time or misrepaired at a rate which is proportional to the square of the damage concentration. In this case, as the dose decreases, the probability of misrepair decreases faster than the probability of repair, and repair becomes a more pronounced feature of the cell response. Since the consequence of unrepaired damage is an important question in determining the effects of low doses of radiation delivered at low dose rates, we have attempted to

determine which of these two types of models is consistent with the response of plateau-phase CHO cells. In the earlier experiments, there was no indication of repair after a 50-rad exposure with a 24-hour split dose or plating delay; in fact, immediate plating resulted in survival slightly above control and delayed plating in survival slightly below the control value.

We now understand the cause of measured survival which exceeds the control. Because of the high surviving fraction following these low doses, a large number of dishes of plateau-phase cells must be irradiated to provide satisfactory precision at each point. Typical experiments involved irradiation of 80 to 120 dishes. These dishes were kept, with caps loosened, in an incubator with active temperature, humidity, and CO₂ control. Originally, groups of dishes receiving the same treatment were processed together and were followed by the group receiving the next treatment. When this procedure was altered so that one dish receiving each treatment was processed and the sequence was then repeated 20 times, a strong dependence of survival on the time of day emerged (Figure 1). This was entirely unexpected because these were 13-day-old

FIGURE 1. Comparison of Survival for Four Different Treatments as a Function of Time of Trypsinization. Upper panel: tissue culture flasks loosely capped in incubator with CO₂ and humidity control. Lower panel: flasks tightly capped in the same incubator. In both cases, the incubator door was opened briefly every 10 to 15 minutes.

plateau-phase cultures; careful study of their growth had shown them to be noncycling but with a high and constant viability until about their 17th day in culture. When this experiment was repeated with the minor variation that the caps of all dishes were tightly sealed at the beginning of the day of the experiment, the survival was essentially constant throughout the day. Thus we conclude that the frequent opening of the incubator to remove dishes for irradiation altered some characteristic, probably the CO₂ concentration, in a way that gradually reduced the plating efficiency of the cultures.

The results of the repair studies at low doses, even after correcting the problem of variation in plating efficiency, still show no repair during either a split-dose interval or after plating delay (Table 1). In fact, these treatments, which enhance survival at higher doses, seem to reduce the survival at low doses. This result would be expected if the probability of misrepair was

proportional to the damage concentration and if the probability of lethality due to sublethal damage interaction depended on the square of the damage concentration. However, it is much too early to draw any conclusions about these mechanisms. More precise measurements will be needed to determine whether a repair interval results in constant or slightly reduced survival. However, it is now clear that the increase in effectiveness of delayed plating repair at low doses, predicted by models incorporating binary misrepair, does not occur in these cells.

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TABLE 1. Observed Repair Under Various Exposure Conditions. (Flasks sealed at start of experiment.)

Treatment	Mean	s	CV
Control	1.00×10^0	$\pm 9.87 \times 10^{-2}$	9.87%
0.50 Gy (prompt plating)	9.53×10^{-1}	$\pm 1.51 \times 10^{-1}$	15.8%
0.50 Gy (24-h plating delay)	8.86×10^{-1}	$\pm 1.08 \times 10^{-1}$	12.3%
0.25 Gy (24-h interfraction interval)/0.25 Gy (prompt plating)	8.80×10^{-1}	$\pm 1.09 \times 10^{-1}$	12.2%

MULTIPLE SPLIT-DOSE REPAIR IN PLATEAU-PHASE MAMMALIAN CELLS

J. M. Nelson, L. A. Braby, and N. F. Metting

Repair of ionizing radiation damage is observed either as the time-dependent removal of radiation-induced alterations in cellular constituents or as changes in the probability of survival of individual cells. The removal of macromolecular alterations is suggestive of mechanisms leading to changes in cell survival probability and several such processes have been observed in bacteria (Town, Smith, and Kaplan 1973). However, even though each has its own characteristic repair rate and other distinguishing characteristics, it has proved difficult to demonstrate unambiguous links between these processes and changes in reproductive integrity leading to the observable enhancement of cell survival. Changes in survival probability are observed in

split-dose and delayed-plating (or liquid-holding) experiments. These changes are certainly responsible for the shoulder observed in dose-response curves and for what we refer to as the dose-rate effect.

The increase in survival resulting from splitting a dose into two or more fractions separated by variable time intervals was first observed in *Chlamydomonas reinhardtii* (Jacobson 1957), and then investigated in considerable depth using mammalian cells (Elkind and Sutton 1959). The change in survival observed was originally assumed to result from repair of innocuous radiation-induced lesions, which were not lethal themselves but which could become lethal if they combined or interacted with additional damage. These lesions were referred to as "sublethal lesions" and their repair termed "sublethal damage repair." Another type of cellular repair was suggested by liquid-holding experiments with prokaryotic cells

(Powers 1962) and was later observed in plateau-phase mammalian cells by holding them in "conditioned" medium for a variable time before reinitiating active growth by trypsinization and refeeding. This type of repair was thought to function on damage that is potentially lethal; that is, damage that would result in the reproductive death of the cell unless it was repaired. It is now evident that these two types of experiments are not sufficient to distinguish between these hypothetical types of damage, because potentially lethal damage repair would also occur during the interval between doses in a split-dose experiment, and could be partially responsible for the observed effect. Consequently, these simple operational definitions of sublethal and potentially lethal damage, based on the types of experiments in which they are observed, are no longer adequate, and newer definitions based on the consequences of the unrepaired damage remaining in the cell are frequently utilized.

The interpretation of experimental results in terms of repair rates and efficiencies is generally based on mathematical models of cellular response. These models are, in turn, based on assumptions about biochemical mechanisms. In many cases, different models based on quite different assumptions lead to nearly identical expressions for the amount of damage remaining. Consequently, survival results of typical experiments are not capable of distinguishing between them. This may be illustrated by the fact that the assumption that all damage is potentially lethal or the assumption that some of the damage is sublethal both provide identical expressions for survival at high dose rates (Curtis 1985). It may require an extensive study of different models and more careful design of critical experiments to show that a particular mechanism is or is not important in a specific situation. We have been studying several models in this way, using plateau-phase CHO cells, but this work is far from completed. Until specific types of mechanisms have been eliminated, many different models can be used to interpret most experimental results.

Because of the importance of repair to the overall response of irradiated cells we have undertaken a study of repair rates in plateau-phase CHO cells as the first step of an extensive study of this system. We have used split-dose experiments with starved plateau-phase CHO cells and investigated the kinetics of repair in terms of enhancement

of reproductive survival. We find it convenient to analyze our data using the accumulation of damage model developed by Roesch (1967, 1978) because this model provides a means of quantifying characteristic repair rates for each process involved. The accumulation model assumes that some initial products of radiation injury are sublethal (innocuous unless they have interacted with additional damage) and that these sublethal lesions are repairable; it is the repair of these sublethal lesions that is responsible for split-dose effect. Results of these split-dose experiments are summarized in Figure 1, where two distinct components of repair are identified. The mean repair time of each will be the negative reciprocal of a line fitted to data plotted as seen in this figure (see Roesch 1967, 1978). The two processes shown in Figure 1 have mean times of about 53 minutes and about 18 hours, respectively.

By using different total doses (Figure 2), we find that both the rates at which these two processes remove damage, and the fraction of damage which each removes, appear independent of the initial amount of damage, i.e., independent of dose. However, the total amount of damage repaired seems to be approximately proportional to the dose. This dose dependence is not consistent with simple models of ionizing radiation damage and repair. Specifically, if the damage were sublethal, the fraction of the cells inactivated should be proportional to the square of the dose. If all damage were potentially lethal and one repair system were to become saturated at a lower dose, the fraction of the population surviving as a result of this process would be constant, i.e., independent of dose. However, the observed proportionality with dose appears to be consistent with models which assume 1) only a portion of the initial damage is directly accessible to the repair systems, or 2) the initial damage is composed of a mixture of potentially lethal and sublethal lesions which are repaired by processes sharing a common rate-limiting step.

These results were reported in a manuscript entitled "Multiple Components of Split Dose Repair in Plateau Phase Mammalian Cells," submitted to Radiation Research.

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FIGURE 1. Split-Dose Survival Data from Irradiated Plateau-Phase CHO Cells, Plotted as $\ln(Sm/S)$ Against the Time Between Irradiations. Cells were irradiated with two equal fractions of 4 Gy each, and plated immediately following the second irradiation. When plotted as $\ln(Sm/S)$ against time on a log scale (Sm/S is the ratio of the maximum survival were complete repair to have taken place to the survival at a given interfraction interval; see Roesch 1978), two components of repair can be distinguished. All data are plotted in the upper frame, but the line is fitted only to the longer-interval data; specifically, intervals greater than 4 h. In the lower frame are plotted the net differences between individual short-interval data and the corresponding values of $\ln(Sm/S)$, extrapolated from the longer-interval data. The mean repair time, $\langle \tau \rangle$, is the negative reciprocal of the slope of the fitted line, in each case.

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FIGURE 2. Adjusted Split-Dose Survival from Plateau-Phase CHO Cells Irradiated at Two Different Doses. The upper curve shows survival after a total dose of 4 Gy; the lower curve, after 8 Gy, in each case given in two equal fractions.

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• Radiation Biophysics

Specific cellular radiobiology studies are often required to test aspects of the mathematical models developed in the Radiation Dosimetry program. These studies are designed to determine whether specific mathematical expressions, which characterize the expected effect of biochemical mechanisms on observable biological responses, are consistent with the behavior of selected cell lines. Since these tests place stringent requirements on the cellular system, special techniques and culture conditions are required to reduce biological variability to a minimum. Cells not progressing through the cell cycle are needed in order to study the effects of dose protraction, and cells in specific parts of the cell cycle are required in order to determine the changes in biochemical mechanisms which are responsible for the cell cycle effect. The use of these specialized cell populations, which have been carefully characterized, is providing data on the extent of repair following low doses and on the changes in the types of damage that can be repaired as the cell progresses toward mitosis.

RADIATION DAMAGE REPAIR BY SUBPOPULATIONS OF STATIONARY-PHASE CHO CELLS SEPARATED BY CENTRIFUGAL ELUTRIATION

J. M. Nelson, N. F. Metting, and L. A. Braby

The sensitivity of cells to radiation (expressed as loss of reproductive capacity) is strongly dependent on the position of the cells within the cell cycle. Similar effects have been seen for mutation and other endpoints, although the times of greatest sensitivity may be observed at different points of the cycle. Since tissues and organs of living animals have characteristic cell division rates which can be perturbed by environmental changes, cell cycle dependence of cellular damage is an essential component of models which are generated in an attempt to correlate the effects in animals with cellular data. Cell cycle variations also provide additional data which may help elucidate the mechanisms controlling cellular damage. Since we have observed two repair rates in plateau phase CHO cells and since these cultures include cells held in various parts of the cycle, it is natural to suspect that the changes in sensitivity may be caused by different repair processes functioning at different parts of the cell cycle. Alternative explanations might include 1) that the amount of relevant damage produced per unit dose may change as a result of the changing configuration of the DNA within the nucleus or 2) that the fraction of the damage which is irreparable may change, possibly due to an effect on the accessibility of the damage to the repair process.

Last year we reported on the design of an experiment to determine if the two repair rates observed in plateau phase CHO cells are characteristic of cells in different

parts of the cycle, or if they occur simultaneously in cells of any age (Nelson et al. 1987). These experiments utilize centrifugal elutriation methods to obtain populations enriched with cells of specific ages.

Centrifugal elutriation separates particles of near-equal densities on the basis of cross-sectional area. Since cells of the same age (i.e., position in the cell cycle) have similar physical characteristics, specifically size and density, this method serves as a convenient tool for isolating cells of a given age. The correlation between DNA content and cell size is not perfect, but elutriation procedures are still satisfactory to produce populations significantly enriched in cells of one cell-cycle age or another.

Stationary-phase CHO cultures produced in this laboratory contain about 83% G(1)-phase cells. The remainder of the population is distributed between both G(2)-phase (~7%) and S-phase (~10%). Using this as a starting population, we have been able to enrich the G(1) and non-G(1) [G(2)+S] phase fractions to a considerable degree. We have also been able to separate G(2)-phase and S-phase cells nearly completely from G(1)-phase cells, and enrich the latter from about 83% to 99%, a nearly pure population. This is much better than we have been able to do with the S-phase or G(2)-phase cells, which can be enriched to only slightly better than 50%. This is about a threefold increase in concentration but the resulting population is still contaminated with nearly 50% G(1)-phase cells.

Split-dose survival following very short intervals is equivalent to, or at least approaches, simple survival. Therefore, survival differences for very short

interfraction intervals should reflect differences in the sensitivity of cells irradiated at different times in the cell cycle. In contrast, a change in the repairability or in the fraction of irreparable damage in the different fractions would result in a separation of the survival curves when the doses were separated by longer intervals (e.g., 6 hours or more).

Generally, when plotted as a fraction of time between doses, the longer-interval survival data from infraction cells, enriched G(1)-phase fractions, or moderately enriched G(2) + S-phase fractions are not significantly different. Consequently, at this time we find no striking differences in the total repair, indicating that damage is removed at generally the same rate and with essentially the same efficiency throughout the cycle. This suggests that changes in the production of damage are more likely responsible for the changes in sensitivity.

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- IMPLEMENTATION OF ADDITIONAL CELL TYPES FOR TRANSFORMATION STUDIES
- J. M. Nelson, N. F. Metting, and L. A. Braby
- We look forward to running several experiments which will require the use of cell transformations as the endpoint of choice. We have studied transformation in vitro, using the C3H 10T1/2 (Clone 8) mouse cell line that we obtained from the American Type Culture Collection (ATCC CCL226) in 1986. This primary mouse cell line was isolated many years ago from several tissues from an embryonic C3H mouse and characterized by Reznikoff, Brankow, and Heidelberger (1973). In the past year, we have characterized its growth kinetics; these studies have been described by N. F. Metting in last year's Annual Report (1987).
- Although this cell line has been used for transformation studies by many workers, particularly for neutron RBE studies (Borek 1978; Hall 1982), there are many difficulties associated with its use. The techniques for maintaining the cell line and for its application to determining transformation frequencies follow those originally published by Reznikoff et al. (1973). These procedures are straightforward, but it has been recognized that the ability of the cells to exhibit transformation is also a function of the type of calf serum used in the growth medium. Because of the difficulty of seeing unstained cell monolayers, identifying individual cells, and even locating developing clones, the process of evaluating viable (live, unstained) cultures is slow and tedious. When it becomes necessary to select viable cells from such a clone, the procedure is exasperatingly difficult and very time consuming. Furthermore, because of these difficulties, we do not believe that automation of the procedures would be cost effective.
- Our experience with 10T1/2 cells, the cell line generally used for such experiments, indicates that these cells are not suitable for our studies, and we are looking elsewhere for alternatives. We have recently made arrangements to obtain two additional cell lines recently developed by G. W. Barendsen of the Radiobiological Institute TNO in the Netherlands (Barendsen 1985). These cell lines were developed specifically for their application to the transformation assay, and we believe they might be much

better suited for this purpose than the C3H 10T1/2 cell line. One of these, the NBCH-3 cell line, was derived from a clone which developed spontaneously in a primary cell culture of tissues from a newborn Chinese hamster. Like the 10T1/2 cells, this clone originated from undefined tissues of somewhat mixed origin, so the specific cell type is uncertain. The assay procedure to be used with this cell line is the same as that for the C3H 10T1/2 cells; however, clonal development and morphology are considerably clearer. In addition, another cell line, denoted WAGR-2, was also derived in Barendsen's laboratory from the tissues of a newborn Wistar rat. The origin of the cells is again uncertain, but the procedures used for determining transformation frequencies with this cell line are essentially the same as for C3H 10T1/2 cells. Use of one or both of these new cell systems for our transformation experiments should not only increase the capabilities of the studies, but their use should make the assay both more accurate and simpler to perform. We also expect that video-recognition techniques can someday be applied to this transformation assay, using the new cell systems.

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THE EFFECT OF OXYGEN ON THE PRODUCTION OF SUBLETHAL DAMAGE

L. A. Braby, J. M. Nelson, and N. F. Metting

The response of cells to the concentration of oxygen in their cytoplasm during irradiation has received a great deal of attention. We found, in cells which were hypoxic but which did not show the full expected oxygen enhancement ratio, that the sparing effect of a split-dose interval of a few hours was eliminated, but that effects of longer intervals or a plating delay were not changed (Nelson et al. 1986). Because this effect depends on the oxygen concentration at the time of irradiation, but is uninfluenced by the concentration during the interval between doses, we believe that it indicates that two different radiation chemistry reactions, one requiring oxygen and the other not, are responsible for the damage that is repaired at the different rates. These results are consistent with many other cell experiments (Alper 1984) and even with some *in vivo* studies (Phillips and Ainsworth 1969), but there are other studies, including some with another Chinese hamster cell line, where oxygen concentration had no effect on repair, but did interfere with cell growth (Elkind et al. 1964). It is evident from these results, taken as a whole, that the processing of radiation damage by the cell is a crucial feature affecting the final outcome of irradiation. It is also evident that this process is energy dependent, and that in some situations anoxia interferes with normal metabolism and thus interrupts energy-dependent repair pathways. In other systems, for example the starved plateau-phase cells we have used, the level of metabolism needed for repair of radiation damage is maintained under moderately hypoxic conditions. The differences in the metabolism of cells in these situations may provide valuable clues to the nature of the processes governing the function of different repair pathways *in vivo* and *in vitro*.

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PRODUCTION OF PERFUSED CULTURES OF CHINESE HAMSTER OVARY CELLS

J. M. Nelson, N. F. Metting, and L. A. Braby

Neoplastic tissues in vivo frequently contain stationary components which have been arrested by natural processes. These processes include, but are not limited to, glucose or other nutritional shortages, lack of oxygen, and lack of space. Additionally, many normal tissues also enter stationary phase, generally as a consequence of contact inhibition and other mechanisms, although regulation of cell growth by such mechanisms is not clearly understood. For example, normal hepatocytes in vivo apparently enter a noncycling "resting" state in response to contact inhibition and extra-cellular control mechanisms; the same is true of many other tissues. Of particular importance is the fact that when normal tissues stop cycling in vivo, they do so at specific, determined positions within their cell cycle. Specific stopping points, or gates, are probably associated with the required functional steps that will be taken when the cells are once again stimulated into cycle. Most resting cells in vivo stop cycling in G(1) phase, immediately following mitosis, e.g., hepatocytes and hemopoietic stem cells. Others, however, like the foot pad epithelium in some rodents, stop cycling in their post-mitotic G(2) phase and begin synthesis immediately upon re-entry into the cycle.

Under normal in vitro growth conditions, most mammalian cells progress rapidly through their life cycle with a generation time on the order of 14 to 18 hours. As radiation sensitivity varies with cell age, survival following a given dose also changes considerably with this progression. In order to perform kinetic studies in vitro from which characteristic repair times can be quantitated, the radiation sensitivity of the population at risk must not be allowed to vary significantly during the course of the experiment. In some cell systems, e.g., the eukaryotic green alga *Chlamydomonas reinhardtii*, this is not a problem (Nelson et al. 1980). Cultures of this cell are spontaneously brought into a synchronous G(1) state, which lasts for 8 to 10 hours, during which radiation sensitivity changes only slightly. However, in mammalian cell systems this is a serious problem which normally prevents quantitation of such parameters.

Several years ago we discovered that Chinese hamster ovary (CHO) cells could be put into stationary phase by nutritional deprivation, at which time DNA synthetic activity, as determined by (3)H-TdR labeling index and both (3)H-TdR and (125)I-UdR cellular uptakes, was depressed between 2 and 3 orders of magnitude (Nelson et al. 1984). We have used these cells for most of our repair-kinetics studies. We also discovered that the population density plateau could also be achieved by other means, such as by feeding, where further growth was prevented by high density contact inhibition. Generally this is accomplished by feeding the cultures on a routine and frequent schedule, such as every other day or three times a week. However, populations produced in this way were found to be considerably different from those produced by nutritional deprivation, i.e., starvation (see Table 1).

Starved populations exist under a great deal of nutritional stress which may cause them

TABLE 1. Phase Distribution in Exponential, Fed, and Starved CHO Cultures

	G(1)	S	G(2)	M
Exponentially Growing	4.5 h 30%	7 h 55%	1.5 h 10%	1 h 5%
Starved Plateau-Phase	~3500 h 83%	~400 h ~10%	~300 h ~7%	-- 0%
Fed Plateau-Phase	60 h 40%	70 h 40%	10 h 20%	1 h <1%

to not be as representative of normal tissue in vivo as we would like. In contrast, fed populations are believed to have been arrested by "contact inhibition," a poorly understood phenomenon which may lead to their having notably different repair potentials. At the time that we evaluated the starved plateau-phase CHO cells in detail, we had also noticed that intermittently fed populations never stop cycling at all. Rather, their growth activity, as defined by DNA synthesis, appeared to oscillate between a "stimulated" growth state and the starved condition, apparently as a consequence of the intermittent nature of the feeding process. We believed that if the cultures could be fed continuously, then the cell density might reach a considerably higher level and the cells arrested by contact inhibition alone. Perfused cells should also be more representative of resting cells in vivo because they would not be stressed with respect to nutrient availability. Under these circumstances, we should expect cells to exit the cycle (enter resting phase) at only one stage, probably just before or after mitosis. This would yield larger populations of better synchronized cells.

To test this possibility, we have attempted to produce plateau-phase cultures of perfused CHO cells using a very low seed peristaltic pump to supply a continuous infusion of nutrient medium to each individual plate. These experiments were met with considerable difficulties. It appears that the demands of this technique exceed the capability of the best commercially available pumping equipment. It also seems unlikely that satisfactory pumping techniques can be developed for continuous perfusion, at a reasonable cost. Consequently, alternative solutions involving the automated frequent feedings, e.g., between 1 and 4 times per day, are currently being investigated.

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• Modeling Cellular Response to Genetic Damage

This project investigates the mechanisms of cellular response to physical and chemical carcinogens that are known to induce damage in deoxyribonucleic acids (DNA). Particular attention has been given to the effect of intracellular pools of deoxynucleotide triphosphates (dNTPs), precursors used in DNA synthesis, on the induction of mutations in Chinese hamster ovary (CHO) cells. Our results suggest that dNTP pools regulate the rate of DNA synthesis and this regulation can dramatically affect the mutagenesis of DNA-damaging agents, probably by altering the extent to which damage is repaired before replication. We observed that an increase in dNTP pools can restore a normal mutation-induction response to a repair-deficient mutant strain of CHO-K1 cells.

DEOXYCYTIDINE MODULATES THE SENSITIVITY OF A REPAIR-DEFICIENT CHO CELL LINE TO UV LIGHT

J. H. Miller, C. H. Newman, and P. Jeggo*

The role of deoxynucleoside triphosphate (dNTP) pools in the response of mammalian cells to radiation and chemical exposure has been investigated intensively in recent years (Newman and Miller 1985a). Table 1 shows that the presence of 2 mM deoxycytidine (CdR) in the growth medium dramatically increases pools of deoxycytidine triphosphate (dCTP) and deoxythymidine triphosphate (dTTP) in a Chinese hamster ovary cell line (CHO-K1) and a radiation-sensitive mutant (xrs-5) derived from it. Since dCMP deaminase activity is high in both cell lines, it is not surprising that CdR increases the size of both dCTP and dTTP pools (Newman and Miller 1985b). In neither medium do we observe a difference between parent and mutant with respect to the size of dCTP or dTTP pools.

We have investigated the correlation between these dNTP pool changes and the effects of 2 mM CdR on the response of the cell lines

to ultraviolet (UV) light and x-ray exposure. Figure 1 shows that UV mutagenesis of xrs-5 is significantly reduced by the presence of CdR while mutation induction in the parent cell line is unaffected. Similarly, Figure 2 shows that the reduction in cell killing by UV light when the growth medium contains 2 mM CdR is greater for xrs-5 than for the parent cell line. Figure 3 shows that the presence of 2 mM CdR has no effect on the sensitivity of either cell line to killing by x-rays.

The observation that 2 mM CdR reduces the UV sensitivity of xrs-5 but has no effect on its x-ray sensitivity implies that either xrs-5 is a double mutant or that some of the damage induced by UV exposure is repaired similarly to x-ray-induced damage. For example, removal of UV-induced photo products from DNA by nucleotide excision repair may lead to the induction of DNA double-strand breaks (DSBs) due to the overlap of repair patches on opposite strands. Since xrs-5 is deficient in DSB rejoining, this is a possible mechanism for the UV sensitivity of xrs-5. If the presence of 2 mM CdR reduces the length of repair patches, this could explain the reduction in the UV sensitivity of xrs-5 without changing its x-ray sensitivity. The possibility that xrs-5 is a double mutant is being investigated

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TABLE 1. Cellular Deoxycytidine Triphosphate and Deoxythymidine Triphosphate Pool Concentrations and Deoxycytidylate Deaminase Activities

Cell	Medium	dCTP ^(a)	dTTP ^(a)	dCMP deaminase ^(b)
xrs-5	standard	1.63 ± 0.33	2.55 ± 0.52	24.5
	+ 2mM CdR	4.03 ± 0.80	6.12 ± 0.2	27.2
CHO-K1	standard	1.19 ± 0.22	3.06 ± 0.63	25.8
	+ 2mM CdR	4.42 ± 0.82	5.65 ± 1.2	22.0

(a) Expressed as picomoles per µg of DNA + S.D. (at least six determinations).

(b) Expressed as nmol dUMP formed/min µg protein⁻¹, x 10⁴.

FIGURE 1. Effect of 2 mM CdR on Induction of Resistance to 6-Thioguanine by Exposure to UV Light in the Repair-Deficient Mutant *xrs-5* and Its Parent Cell Line CHO-K1

through the use of revertants of the x-ray-sensitive phenotype.

The relationship between the pool effects of 2 mM CdR and its reduction of the UV sensitivity of *xrs-5* is uncertain. Clearly, we cannot attribute this effect to imbalance in dNTP pools since Table 1 shows that the ratio of the dCTP pool to the dTTP pool remains constant at 0.65 for *xrs-5* when CdR

is added to the medium but changes significantly (0.39 to 0.78) for the parent CHO-K1 cell line. If the changes in UV sensitivity of *xrs-5* are related in any way to the changes in dCTP or dTTP pools, then *xrs-5* and CHO-K1 must respond differently to elevated dCTP and dTTP pools. This suggests that *xrs-5* may have a mutation that affects DNA polymerase alpha. In support of this hypothesis, Figure 4 shows that the patterns

FIGURE 2. Effect of 2 mM CdR on Killing by UV Light of the Repair-Deficient Mutant xrs-5 and Its Parent Cell Line CHO-K1. Ordinate is the negative of the logarithm of the surviving fraction.

of DNA replication in xrs-5 are affected by the presence of 2 mM CdR, while the this is not the case for its parent cell line.

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FIGURE 3. Dose Responses for Cell Killing by X-Rays in the Presence (open symbols) and Absence (closed symbols) of 2 mM CdR for Repair-Deficient Mutant *xrs-5* (squares) and Its Parent Cell Line CHO-K1 (circles)

FIGURE 4. Alkaline-Sucrose Gradient Profiles of DNA from [^3H]TdR Pulse-Labeled *xrs-5* (squares) and CHO-K1 (circles) Cells Grown in the Presence (open symbols) or Absence (closed symbols) of 2 mM CdR. The post-labeling growth period ("chase") for *xrs-5* and CHO-K1 was 5 and 10 minutes, respectively. X denotes profile of [^{14}C] TdR-labeled chromosomal DNA.

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