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**Part 4 Physical Sciences
February 1987**

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

**Pacific Northwest Laboratory
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L.H. Toburen and Staff

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Pacific Northwest Laboratory
Richland, Washington 99352

PREFACE

This 1986 annual report from Pacific Northwest Laboratory (PNL) to the Department of Energy (DOE) describes research in environment, health, and safety conducted during fiscal year 1986. The report again consists of five parts, each in a separate volume.

The five parts of the report are oriented to particular segments of our 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 1986 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	R. E. Wildung, Report Coordinator K. A. Borgeson and S. G. Weiss, Editors
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: Nuclear and Operational Safety Program Managers - L. G. Faust W. E. Kennedy, Jr. J. M. Selby B. L. Steelman	L. G. Faust, Report Coordinator S. K. Ennor, Editor

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.

A major effort was in response to OHER's request for assistance in collecting information on the release of radioactivity from the nuclear power plant accident at Chernobyl. Environmental and chemical scientists participated in collecting and analyzing atmospheric and environmental samples in Sweden, over the Pacific Ocean, along the northwest coast of North America and along the path of the radioactive plume as it passed across the United States.

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 last year are:

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FOREWORD

Part 4 of the Pacific Northwest Laboratory Annual Report for 1986 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 1985. The reader should contact the principal investigators named or examine the publications cited for more details.

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• Chemical Basis for Biological Response to Energy-Related Organics

Studies of the chemistry of energy-related organic compounds in biological systems have focused primarily on the disposition of polycyclic aromatic hydrocarbons (PAHs) and polycyclic aromatic amines (PAAs) in mammalian systems. Knowledge gained from these studies will be applied to obtaining a more reliable estimate of risk from exposure to energy-related organics.

These fundamental studies of biological effects of energy-related organics are funded by the Office of Health and Environmental Research (OHER).

DETERMINATION OF ADDUCTS OF BENZO[A]PYRENE TO DEOXYRIBONUCLEIC ACID USING GAS CHROMATOGRAPHY/MASS SPECTROMETRY TECHNIQUES

E. K. Chess, B. L. Thomas, D. L. Springer, D. B. Mann, D. J. Hendren, and R. M. Bean

Adducts to deoxyribonucleic acid (DNA), formed from metabolites of polynuclear aromatic compounds, are relatively persistent and correlate with bioresponse (carcinogenicity) (Brookes and Lawley 1964; DeSerres, Gledhill, and Sheridan 1985; Sims et al. 1974). Therefore, qualitative and quantitative analyses of adducts in the DNA of individuals may provide valuable information on recent exposure to carcinogenic hydrocarbons. Furthermore, the ability to detect adducts in a large segment of a population may have significant epidemiological significance. However, adducts are difficult to analyze because they are formed at extremely low concentrations. Adducts in the placenta of human cigarette smokers have been detected in concentrations as low as 1 adduct per 10^{10} DNA base units (Everson et al. 1986), 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. 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.

The basic thrust of the analytical development at PNL is to isolate the DNA, liberate the adducted hydrocarbon residue from the DNA with acid hydrolysis, and prepare derivatives of the hydrolyzed species that will enhance its detection, quantitation, and characteri-

zation using gas chromatography/mass spectrometry (GC/MS). We have initiated the development of the necessary techniques using benzo[a]pyrene (BaP) and its metabolites. Samples of DNA adducts of radiolabeled BaP have been prepared for study by reacting DNA isolated from calf thymus with benzo[a]pyrene-7,8-diol-9,10-epoxide (the ultimate carcinogenic form of BaP). The identity of the synthesized adduct was confirmed by mass spectrometry. Other DNA/BaP samples have been prepared by painting the skin of mice with radiolabeled BaP. These methods will be used to prepare adducts from other polynuclear aromatic hydrocarbons in the future.

The liberation of the adducted hydrocarbon residue (as the tetrahydrotetrol) from a BaP/DNA adduct has been shown by Rahn et al. (1982) to be facile using 0.1 N hydrochloric acid at 80°C for 6 hours. We have found that good recoveries of the tetrols are obtained from the hydrolytic conditions in the presence of DNA using a reverse-phase cleanup column to isolate the tetrol. However, we have also determined that the hydrolytic conditions cause epimerization of the tetrol, presumably at about the 10 position. The results of experiments in which pure tetrol isomers were exposed to acid hydrolysis conditions are shown in Figure 1. About 12% of the principal product formed from the anti-BaP diol epoxide (I-1, Figure 1) is isomerized to the I-2 form during hydrolysis; and the II-1 isomer expected from the Syn-BaP diol epoxide is extensively isomerized to the II-2 isomer, with a small portion forming an as yet unidentified compound (Figure 1). These experiments have shown that while the recoveries of tetrols under hydrolytic conditions are acceptable, information about the conformation of the adduct when attached to the DNA is lost during the cleavage step.

	<u>I-1 (Anti)</u> <u>r-7, t-8, 9, c-10</u>	<u>I-2 (Anti)</u> <u>r-7, t-8, 9, 10</u>	<u>II-1 (Syn)</u> <u>r-7, t-8, c-9, t-10</u>	<u>II-2 (Syn)</u> <u>r-7, t-8, c-9, 10</u>	<u>Unknown</u> <u>Compound</u>	<u>%</u> <u>Recovery</u>
I-1	88%	12%	--	--	--	71 ± 4
I-2	89%	11%	--	--	--	87 ± 7
II-1	--	--	15	81	4	88 ± 12
II-2	--	--	15	79	7	85 ± 14

FIGURE 1. Isomerism of BaP Under Acid Hydrolysis Conditions, and Recovery of Tetrols from the Hydrolysis Treatment (diagrams show tetrol ring only)

Once the tetrols are removed from the DNA polymer and isolated, they are reacted with reagents that make them sufficiently volatile to permit their analysis by GC/MS methods. Initial development work on GC/MS analysis has focused on methods for analyzing the BaP metabolites, 3-hydroxy-, 7,8-dihydro-7,8-dihydroxy-, 7,8,9,10-tetrahydro-7,8,9-trihydroxy-, and 7,8,9,10-tetrahydro-7,8,9,10-tetrahydroxy-BaP. These compounds are light and heat sensitive, and require derivatization of the polar hydroxyl groups before analysis. Several types of derivatives (permethyl, peracetyl, and trifluoroacetyl) have been prepared for each metabolite and have been tested to determine which type of derivative will offer the best performance in terms of stability and sensitivity for the analysis of trace levels isolated from biological systems. Electron impact (EI), methane chemical ionization (CI), and electron-capture negative-ion chemical ionization (NICI) have been evaluated to determine which ionization mode offers the best overall sensitivity and selectivity.

Permethylated derivatives were prepared and analyzed by GC and GC/MS using relatively short (<10 m) fused-silica capillary columns. Molecular ions were found for all modes of ionization, but the CI spectra generally contained more extensive fragmentation than did the EI spectra. The EI mass spectra provided the most diagnostic fragmentation information related to the structure of the

ion. For example, the prominent molecular ion (m/z 376) of the permethylated tetrahydrotetrol (Figure 2) undergoes a retro-Diels Alder reaction, expelling a molecule of 1,2-dimethoxyethene containing C-8 and C-9 from the BaP ring structure and resulting in the fragment ion at m/z 288. This ion then loses a methyl radical, primarily originating from the methoxy group attached to C-10 (BaP numbering system), to produce the base peak fragment ion at m/z 273. This fragmentation pathway has been confirmed by deuterium labeling experiments.

The peracetate and trifluoroacetate derivatives were successfully prepared using standard derivatization procedures with acetic anhydride or N-methyl-N-trimethylsilyltrifluoroacetamide, respectively, for all metabolites except the 7,8-dihydrodiol, which appeared to either dehydrate under the reaction conditions or decompose during analysis, resulting in chromatograms which contained peaks for both a derivatized and underivatized mono-hydroxy-BaP. Some peracetate and trifluoroacetate derivatives appeared to undergo thermal decomposition during GC and GC/MS analysis using conventional splitless injection. On-column injection has been shown to improve the analysis both qualitatively and quantitatively by reducing both injector discrimination and thermal decomposition. As anticipated, the peracetate derivatives all display retention times that are several minutes longer than their

FIGURE 2. Mass Spectrum (70 eV) of the Permethyl Derivative of BaP-Tetrahydrotetrol

permethyl analogs, indicating a comparative decrease in volatility. The EI mass spectra for the acetates display the diagnostic loss of acetic acid for acetates attached to a partially hydrogenated aromatic ring, and the loss of ketene for acetates attached to a fully aromatic ring. The CI and NCI mass spectra for the tetrahydrotetrol acetate and perfluoroacetate display very weak molecular ions and strong peaks for fully aromatic fragment species.

The trifluoroacetate derivatives were found to decompose after only a few hours in solution, but the peracetate derivatives were stable in solution for several days. The permethyl derivatives were found to be the most stable of the three types, and a several-milligram quantity of permethyl tetrahydrotetrol was prepared and purified for use as an analytical standard.

Initial experiments to determine the detection limits of the derivatized metabolites indicated that <0.6 pg of permethylated and ~0.05 pg of the peracetylated tetrahydro-

tetrol could be detected during single-ion monitoring (SIM) GC/MS analyses using a mass selective detector. These levels of sensitivity are sufficient to detect the amounts of DNA-bound metabolites isolated from laboratory animals exposed to relatively high levels of carcinogens, and may be sufficient to detect the hydrolyzed metabolites at levels that can be isolated from environmentally exposed systems.

Results from the initial methodology development stages of this assay indicate to us that GC/MS analysis using SIM can provide the specificity and sensitivity required for the quantitative determination of derivatized PAH metabolites isolated from DNA adducts that have been formed as a result of environmental exposure to PAH.

REFERENCES

Brookes, P., and P. D. Lawley. 1964. *Nature* 202:781-4.

De Serres, F. J., B. L. Gledhill, and W. Sheridan, eds. 1985. Environ. Health Persp. 62:3-231.

Everson, R. B., E. Randerath, R. M. Santilla, R. C. Cefalo, T. A. Avitts, and K. Randerath. 1986. Science 231: 54-57.

Rahn, R. O., S. S. Chang, J. M. Holland, and L. R. Schugart. 1982. Biochem. Biophys. Res. Commun. 109:62-68.

Sims, P., P. L. Grover, S. Swaisland, and A. Hewer. 1974. Nature 252:326-27.

Wogan, G. N., and N. J. Gorelick. 1985. Environ. Health Persp. 62:5-18.

DISAPPEARANCE OF BENZO[A]PYRENE AND OTHER POLYCYCLIC AROMATIC HYDROCARBONS FROM MOUSE SKIN IN VIVO

C. W. Wright and D. A. Dankovic

Previous experimental data showed that increased skin tumor initiation activity of complex coal-derived mixtures was correlated with products of higher boiling-point ranges and that the neutral PAH fraction of these complex mixtures was the most active fraction, using skin tumor initiation as a biological endpoint. When added to benzo[a]pyrene (BaP), however, the high-boiling coal-derived mixtures have suppressed the skin tumorigenicity of the carcinogenic parent compound, indicating that the tumorigenic coal-derived mixtures are not additive with BaP carcinogenicity, but appear to be inhibitory. Other experiments have shown that coal-derived complex mixtures inhibit the covalent binding of BaP to mouse skin DNA in vivo, and are competitive inhibitors of BaP metabolism in vitro.

In the present work, the effects of the PAH fraction isolated from a high-boiling coal-derived complex mixture on the rate of disappearance of BaP from mouse skin in vivo were examined. The rate of disappearance from mouse skin was used as an indirect measure of the rate of BaP uptake and metabolic activation by the mixed function oxidase (MFO) system of mouse skin. In addition, the individual rates of disappearance from mouse skin of 12 PAH compounds in the PAH fraction were calculated.

The PAH fraction was isolated from the 800 to 850°F distillate cut from the solvent-refined coal (SRC)-II process. Female CD-1 mice (four per group) were shaved 1 to 2 days before dosing, and the shaved areas were treated with either 25 µg of BaP or 8 mg of the 800 to 850°F PAH fraction (containing approximately 27 µg BaP). At intervals of 1, 2, 4, 8, 16, or 24 hours after dosing, the mice were killed and the dosed areas of skin removed. Time 0 samples were generated by dosing the shaved area of skin on previously killed mice, and immediately removing the dosed skin patch. The skin patches were transferred into 25 mL screw-top glass centrifuge tubes and were digested and then extracted twice with 5.0 mL aliquots of n-hexane. The pooled hexane fractions were concentrated under nitrogen to approximately 4 mL (for the 0 to 4-hour samples) or 1 mL (for the 8 to 24-hour samples).

Rates of disappearance on mouse skin of the dosed BaP and 12 PAH compounds in the dosed PAH fraction were determined from quantitative high-resolution gas chromatographic analysis of hexane extracts. The components quantified in the extracts were identified by retention time and mass spectral information. 2-chloroanthracene and 9-phenylcarbazole were added as internal standards. Instrument calibration was done using a National Bureau of Standards (NBS) standard PAH mixture, SRM 1647, and individual four- to six-ringed PAH compounds chromatographed at eight concentration levels ranging over two orders of magnitude. Extraction efficiencies of the selected PAH and BaP were determined from the time 0 extract by comparison to the concentrations of components in the original dosing solutions. All other time interval PAH recoveries were corrected for extraction losses by normalizing to a 100% time 0 recovery. The natural logs of the percent recoveries of each of the compounds were plotted versus the length of exposure time. The biological half-lives on mouse skin for the dosed BaP and 12 PAH compounds in the dosed 800 to 850°F coal-derived complex mixture PAH fraction were determined from the slopes of the linear semi-log plots.

The half-lives of disappearance on mouse skin for the 12 selected PAH compounds in the PAH fraction isolated from the 800 to 850°F coal liquid are given in Table 1. (The results for these specifically named components also

TABLE 1. Half-Lives on Skin of Benzo[a]pyrene and Selected Polycyclic Aromatic Hydrocarbons in Coal Liquid at 800 to 850°F

Compound ^(a)	Skin Half-Life, ^(b) hours
Pyrene	5.0 ± 0.6
Chrysene	6.5 ± 1.0
Benzo[b]fluorene	6.7 ± 1.2
Benzo[a]pyrene ^(c)	6.7 ± 1.3
Methylchrysene	6.9 ± 1.2
Benzo[a]anthracene	7.3 ± 1.4
4- and/or 6-Methylchrysene	7.4 ± 1.3
Methylchrysene	7.4 ± 1.3
C ₄ -Pyrene	7.6 ± 1.5
Benzo[j] or b]fluoranthene	8.2 ± 1.7
C ₂ -Chrysene	8.7 ± 1.7
Benzo[e]pyrene	8.8 ± 2.0
Benzo[a]pyrene ^(d)	3.0 ± 0.3

(a) Tentative identifications based on retention times and GC/MS data.

(b) Half-life ± two standard errors based on the standard error of the slope.

(c) Determined as a component of the PAH fraction.

(d) Determined as 25 µg applied alone.

include any co-eluting compounds.) These half-lives ranged from 5.0 to 8.8 hours, with the species having the lowest molecular weight exhibiting the shortest half-life. Within this limited data set there appeared to be some association of half-life with the number of rings and with the degree of alkyl substitution of the parent PAH. With the exception of BaP, all the four-ringed parent compounds had shorter half-lives than did the five-ringed parent compounds (although the differences were not necessarily significant). There was also a trend for the increased-alkyl-substituted components to have increased half-lives (indicating a decreased rate of uptake) compared to their parent PAH or less-alkyl-substituted counterparts. Coal-derived complex mixtures have been noted to yield decreased tumorigenic potency when increased concentrations of highly alkylated species were present.

Also given in Table 1 is the half-life of BaP on mouse skin when applied alone. The skin half-life of the 25-µg dose of BaP applied alone was significantly shorter than the skin half-life of a nearly comparable

dose applied with the complex PAH fraction isolated from the 800 to 850°F coal liquid (3.0 ± 0.3 hours, versus 6.7 ± 1.3 hours, respectively), indicating (in an indirect way) an increased rate of uptake and metabolic activation of BaP in the former over the latter case. The increase in skin half-life in the presence of the complex mixture may be due to the competitive inhibition of the metabolism of BaP by other components in the mixture. This apparent inhibition of skin metabolism may be related to the observed inhibition of skin tumor initiating activity of BaP and the observed inhibition of BaP binding in skin when in the presence of complex coal-derived liquids.

CHARACTERIZATION OF NITROSATED COAL LIQUID AT 800 TO 850°F

C. W. Wright and E. K. Chess

The purpose of this study was to chemically characterize a complex, high-boiling coal-derived mixture that was assayed in several biological regimes to determine the fate of the amino-substituted polycyclic aromatic hydrocarbons (amino-PAHs) and the integrity of other chemical classes upon nitrosation. Nitrosation has been noted to reduce significantly the microbial mutagenic response of complex coal-derived materials. Previous studies were performed by nitrosating standard mixtures of representative compounds from the following chemical classes: PAHs, aliphatic hydrocarbons, partially hydrogenated PAHs (hydroaromatics), nitrogen-containing polycyclic aromatic compounds (NPACs), cyano-substituted PAHs, hydroxy-substituted PAHs (hydroxy-PAHs), and alkyl-substituted PAHs. General results from the nitrosation of the standard mixtures indicated that none of the standards were affected by the chemical reaction conditions with the exception of the amino-PAHs and some easily oxidized compounds. Some of the amino-PAH nitrosation products were parent PAH, chloro-substituted PAH, methoxy-substituted PAH (from the methanol solvent), and aromatic ketones. Little conversion to hydroxy-PAH occurred, as was anticipated according to the literature. An easily oxidized compound, 9-methylanthracene, was oxidized to a quinone when nitrosated.

In the present study, the 800 to 850°F distillate from the solvent-refined coal (SRC)-II process was fractionated, by adsorption column chromatography using neutral alumina, into chemical class fractions of aliphatic hydrocarbons (AHs), PAHs, NPACs, and hydroxy-PAHs. Half of the NPAC fraction was nitrosated at room temperature using NaNO_2 and HCl followed by hydrolysis and solvent extraction. Both the nitrosated and non-nitrosated NPAC fractions were refractionated by the above method into AH, PAH, NPAC, and hydroxy-PAH fractions. The refractionated NPAC fractions were further subfractionated, by adsorption column chromatography using silicic acid, into secondary amine (carbazoles), primary amine (amino-PAH), and tertiary amine (azaarenes) subfractions. The amino-PAH subfractions from both the nitrosated and non-nitrosated NPAC fractions were selectively derivatized with pentafluoropropionic anhydride (PFPA). All fraction weights were determined gravimetrically. Fractions and subfractions were analyzed by high-resolution gas chromatography (GC), gas chromatography/mass spectrometry (GC/MS), and low-voltage probe-inlet mass spectrometry (LVMS).

The chemical class fractionation weight percent results for the refractionation of the nitrosated and non-nitrosated NPAC fractions, respectively, were: 0% and 0% AH; 4% and <1% PAH; 82% and 93% NPAC; and 10% and 2% HPAH. The nitrosated material had a higher PAH and hydroxy-PAH composition than its non-nitrosated counterpart. The nitrosated material also had a lower amino-PAH subfraction content than did the non-nitrosated material, as based on the weight percent eluting in this subfraction.

The LVMS analysis results from the derivatized amino-PAH subfraction from both the nitrosated and non-nitrosated materials are shown in Figure 1 (the component with molecular weight 212 is the 2-chloroanthracene internal standard). The masses above approximately 350 atomic mass units (amu) represent the amino-PAH with the added PFPA derivative group (the PFPA group adds 146 daltons to the molecular weight of the non-derivatized primary aromatic amine). The

percent of the total ion current accounted for by masses greater than 350 amu in the non-nitrosated 800 to 850°F NPAC distillate was 35.1%, whereas it was <0.1% in the nitrosated NPAC distillate, indicating a dramatic decrease in amino-PAH composition in the complex mixture upon nitrosation.

The results of the GC and GC/MS analyses of the PAH fractions isolated from the nitrosated and non-nitrosated NPAC fractions are shown in Figure 2. The peaks showing the greatest difference in concentration between the two fractions are labeled with their major component molecular weights. Increased concentrations of relatively lower molecular weight PAH components were present in the nitrosated versus the non-nitrosated material. The molecular weights of the PAH components that showed an increased concentration upon nitrosation of the NPAC fraction corresponded to the molecular weights of the non-nitrosated derivatized amines when a PFPA derivatized amino functionality was added onto the parent compound, as shown in Figure 2 and compared with Figure 1 (the addition of the PFPA derivatized amino group adds 161 daltons to the molecular weight of the parent PAH). These data indicated that some of the amino-PAH compounds were reduced to their corresponding parent PAH upon nitrosation.

Analyses of the hydroxy-PAH fraction and tertiary amine (azaarenes) subfraction showed no detectable differences in the compounds present after nitrosation compared to the non-nitrosated fractions. Two components of mass 167 (tentatively identified as azafluorene isomers; the retention times did not correspond to carbazole) were detected in the non-nitrosated versus the nitrosated material in the secondary amine (carbazoles) subfractions. These compounds would be susceptible to oxidation to ketones under the conditions of the nitrosation reaction, although no evidence of this reaction was detected. No methoxy- or chloro-substituted PAHs were detected in the nitrosated fractions, although they may be present in trace concentrations.

FIGURE 1. Low-Voltage Probe-Inlet Mass Spectra of the PFPA Derivatized Amino-PAH Fractions Isolated from the Non-Nitrosated (a) and Nitrosated (b) NPAC Fractions of the SRC-II Distillate Coal Liquid at 800 to 850°F

FIGURE 2. Gas Chromatogram of the PAH Fractions Isolated from the Non-Nitrosated (a) and Nitrosated (b) NPAC Fractions of the SRC-II Distillate Coal Liquid at 800 to 850°F

Measurement
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Dosimetry

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• 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 CHROMATOGRAPHY AND MASS SPECTROMETRY DEVELOPMENT FOR FOSSIL FUELS

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

Chromatographic methods combined with mass spectrometry 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 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) and can do so with higher efficiencies than liquid chromatography.

Recent work has demonstrated that the performance of the supercritical fluid chromatography-mass spectrometer (SFC-MS) interface depends largely on the fluid expansion step. Detailed studies of the expansion of fluids through capillary restrictors identified several mechanisms whereby nonvolatile analytes could be lost, thus precluding their analysis. As a result, a revolutionary mass spectrometer ion source and interface was designed to address the various loss mechanisms.

The new SFC-MS interface incorporates a mechanically pumped expansion region behind the chemical ionization source repeller electrode. The SFC effluent enters the expansion region through a temperature-regulated drawn fused-silica restrictor. The arrangement allows gas flow rates ~25 times higher (100 to 200 mg CO₂/min) than previous interfaces and provides a significant improvement over direct mechanical pumping of the ion source volume in terms of pumping speed, background contributions, ionization efficiency, and overall sensitivity. The new SFC-MS interface allows injection of the entire effluent of microbore (1 mm inside diameter, i.d.) or 100- μ m-i.d. capillary columns, at linear velocities of up to 25 cm/s for the latter. Splitless injection has thus been made possible for the first time, as was demonstrated for both microbore and capillary columns, allowing quantitation over a wide range of analyte concentration.

The effects of sample flow rate, restrictor geometry, and effluent heating were briefly studied in relation to the transfer and detection of less-volatile and nonvolatile analytes, and a significant improvement was noted for the new interface over previous designs. Improved performance for nonvolatile analytes is attributed to the high fluid density at the capillary exit for high flow rates and short tapered restrictors. Small amounts (<1%) of methanol significantly improved the microbore separations, but had a negligible effect on the capillary column separations for well-deactivated columns. Microbore columns were shown to have greater limitations for pressure programming, and generally superior operation was obtained with capillary columns. Further improvements to this interface are in progress, and its extension to highly polar supercritical

fluids (e.g., ammonia), which are necessary for application to many currently intractable analytical problems, is anticipated.

REFERENCES

- Smith, R. D., W. D. Felix, J. C. Fjeldsted, and M. L. Lee. 1982. "Capillary Column Supercritical Fluid Chromatography/Mass Spectrometry." Anal. Chem. 54:1883.
- Smith, R. D., J. C. Fjeldsted, and M. L. Lee. 1982. "Direct Fluid Injection Interface for Capillary Supercritical Fluid Chromatography-Mass Spectrometry." J. Chromatog. 247:231.
- Smith, R. D., and H. R. Udseth. 1983a. "Mass Spectrometry with Direct Supercritical Fluid Injection." Anal. Chem. 55:2266-2272.
- Smith, R. D., and H. R. Udseth. 1983b. "New Method for the Direct Analysis of Supercritical Fluid Coal Extraction and Liquefaction." Fuel 62:466.
- Smith, R. D., and H. R. Udseth. 1983c. "Direct Mass Spectrometric Analysis of Supercritical Fluid Extraction Products." Sep. Sci. Tech. 18:245.
- Smith, R. D., and H. R. Udseth. 1984. "Direct Supercritical Fluid Injection Mass Spectrometry of Mycotoxins." Biomed. Mass Spectrom. 10:577-580.
- Smith, R. D., H. T. Kalinoski, H. R. Udseth, and B. W. Wright. 1984. "Rapid and Efficient Capillary Column Supercritical Fluid Chromatography with Mass Spectrometric Detection." Anal. Chem. 56:2476-2480.
- Smith, R. D., B. W. Wright, and H. R. Udseth. 1984. "Supercritical Fluid Methods in Analytical Chemistry." In Anal. Spectros. Sym. Ser. 19:375. Ed. W. S. Lyon. Elsevier Scientific Publishing Co., Amsterdam, The Netherlands.
- Yonker, C. R., B. W. Wright, H. R. Udseth, and R. D. Smith. 1984. "New Methods for Characterization of Supercritical Fluid Solutions." Ber. Bunsenges Phys. Chem. 88:908.

• Lasers in Environmental Research

Laser light has unique properties that can be applied to analytical chemistry to improve both sensitivity and selectivity. Pacific Northwest Laboratory is developing laser-based methods for measuring low levels of toxic materials and environmentally hazardous substances. In the last year, we have continued development of continuous-wave double-resonance ionization mass spectrometry, a technique that combines isotopically selective ionization with mass spectrometry to provide improved discrimination against isobaric interferences. We have also studied effects that can occur in some continuous-wave laser experiments, such as anomalous peak-height ratios, and that could adversely influence analytical results.

CONTINUOUS-WAVE DOUBLE-RESONANCE IONIZATION MASS SPECTROMETRY

T. J. Whitaker, B. D. Cannon, G. K. Gerke, and B. A. Bushaw

Continuous-wave (cw) lasers offer two distinct advantages over pulsed lasers in analytical applications; they typically have narrower bandwidths by 3 to 4 orders of magnitude, and they have unity duty cycle. The first property permits isotopically selective excitation of elemental species. The second property can be exploited to improve the overall sensitivity in many types of analysis.

We have developed a technique that employs two cw dye lasers to sequentially excite an atomic species to a high electronic state (Bushaw et al. 1986; Whitaker 1986; Whitaker, Cannon, and Bushaw 1986). The excited atoms are then photoionized by a powerful infrared laser. The cw dye lasers have very high-wavelength resolution and can be tuned to a specific isotope of the target element. In recent experiments on an atomic beam of barium (Ba), we demonstrated efficient ionization of ^{137}Ba with almost 10^5 selectivity over the major natural isotopic component, ^{138}Ba . This selectivity is especially notable because spectral lines in barium isotopes are very close to each other and therefore difficult to resolve. This technique, with its combination of mass selectivity from the mass spectrometer with isotopic selectivity in the laser ionization step, makes possible the analysis of isotopic abundances of 10^{11} and greater.

We have improved the excitation scheme to improve the sensitivity of the technique.

This improvement involves exciting the atom to a higher electronic state and using a CO_2 laser for photoionization. Ionization efficiency is increased because of several factors: longer lifetime of the excited state, greater photon flux from the CO_2 laser, and a higher cross-section for photoionization. Ionization efficiencies of 6% have been observed for atoms in the laser beam. This is significantly better than most ionization techniques used in mass spectrometry (see Figure 1) and provides the added advantage of elemental and isotopic selectivity.

In some cw resonance ionization experiments, it is possible for an atom to undergo several excitation/emission cycles before the excited-state atom is ionized. This can lead to a redistribution of the magnetic substate populations. We have discovered previously unreported effects that were caused by redistribution of the magnetic substate populations. These effects include anomalous peak-height ratios that could introduce errors in the interpretation of data, even if isotopic dilution techniques are used. For example, we have observed that the peak-height ratio of the transitions $(6s6p\ ^1P_1 \rightarrow 6s^2\ ^1S_0)$ to the $F=5/2$ and $F=3/2$ upper hyperfine states in ^{137}Ba increases from about 1.5 to over 4.0 as laser intensity increases. We have used a rate equation model to explain this effect. An interesting implication of this model is that some transition line widths should broaden and others narrow at laser intensities well below saturation. The ^{137}Ba transition to the $F=3/2$ hyperfine state shows such an anomalous broadening by almost 50% above the natural line width. The rate model shows that these undesirable effects can be avoided by proper selection of laser intensities in the experiments.

FIGURE 1. Efficiency and Selectivity in cw Resonance Ionization Process. The ranges of sensitivity and selectivity of various continuous duty cycle ionization methods are shown. EI = electron impact; LEEII = laser-enhanced electron impact ionization; RIMS = resonance ionization mass spectrometry; DRIMS = double resonance ionization mass spectrometry.

REFERENCES

- Bushaw, B. A., B. D. Cannon, G. K. Gerke, and T. J. Whitaker. 1986. "Laser-Enhanced Electron-Impact Ionization Spectroscopy." *Opt. Lett.* 11(7):422-424.
- Whitaker, T. J. 1986. "Isotopically Selective Laser Measurements." *Lasers and Applications* 5(8):67-73.
- Whitaker, T. J., B. D. Cannon, and B. A. Bushaw. 1986. "Lasers in Analytical Chemistry." In *Physical Sciences. Part 4 of Pacific Northwest Laboratory Annual Report for 1985 to the DOE Office of Energy Research.* PNL-5750 PT4, Pacific Northwest Laboratory, Richland, Washington, pp. 11-12.

**Physical Processes
in Radiation
Biology**

Physical Science
at
Union
College

PHYSICAL PROCESSES IN RADIATION BIOLOGY

The Radiological Physics programs address the physical processes of radiation action on biological systems. Through integrated, multidisciplinary studies, the interaction of radiation with matter of biological significance is examined. The resulting information is integrated into predictive models of biological response.

Increased understanding of the physical, chemical, and biological mechanisms of radiation action results in a better understanding of dose-response relationships in terms of quantifiable characteristics of the radiation and the absorbing media. Experimental studies into the fundamental physical properties of energy deposition, energy transport, and cellular response are conducted to provide a data base for developing and testing chemical and biophysical models. Emphasis is on correlating radiation quality and energy transport with the formation of active chemical species; on understanding the mechanisms by which chemical agents interact at the cell level; and on developing models that correlate physical parameters of the radiation, chemical/biochemical interactions, and cellular repair phenomena with observed dose-response relationships.

• Radiation Physics

Initial Interaction Processes

A fundamental knowledge of the mechanisms by which radiation interacts with matter is essential to understanding dose-response relationships in terms of quantifiable chemical and physical processes. Our study of the initial interaction processes in the absorption of energy from a radiation field has concentrated on measuring and interpreting absolute cross sections for the interaction of fast charged particles with atomic and molecular targets. Because ionization is the primary means of energy deposition by fast charged particles, we have emphasized measurements of total, differential, and partial ionization cross sections. Cross sections for the production of electrons differing in ejected electron energy and emission angle are required for detailed track structure information, whereas partial ionization cross sections provide information on the final states of the target atom/molecule. Of particular interest is the determination of cross-section systematics and interaction mechanisms that enable the extrapolation of experimental results into regions where experiments are either impractical or unfeasible. During the past year, we have made progress in three primary areas: 1) the study of mechanisms responsible for production of multiply ionized targets by interactions with fast charged particles, 2) the study of ionization involving clothed charged particles (ions carrying bound electrons such as He^+ , C^+ , etc.), and 3) the theory of ionization for both bare and clothed charged particles. Efforts focused on investigating the interaction pathways leading to multiple processes such as multiple ionization and simultaneous ionization and charge transfer. Doubly differential ionization cross sections were studied for He^+ ions using coincidence techniques to separate target and projectile ionization and for C and O ions to investigate cross section scaling. Theoretical studies emphasized use of the Bethe-Born approximation in extrapolating singly differential electron emission cross sections for both bare and clothed ions. This project continues to benefit from a close working relationship with Dr. S. T. Manson, Georgia State University, Dr. M. E. Rudd, University of Nebraska, and Dr. P. Goldhagen, Columbia University.

TARGET IONIZATION BY PROTON IMPACT

R. D. DuBois

In our attempts to understand proton impact ionization of atomic and molecular targets, we have measured total ion (σ^+) and electron (σ^-) production cross sections (Rudd et al. 1983), cross sections for multiple-target ionization ($\sigma^{\text{I}q}$) without regard to charge transfer (DuBois, Toburen, and Rudd 1984), and multiple-target ionization cross sections ($\sigma^{\text{I}j}_q$) for direct ionization ($i=j$) and for charge transfer ($j<i$) (DuBois 1984). These cross sections have been combined with other information obtained from the literature (e.g., total charge transfer cross sections, inner-shell cross sections, and Auger branching ratio information) to provide a total, consistent picture of target ionization resulting from proton impact. This work is being done in collaboration with S. T. Manson.

One of the aims of this effort is to show the relationship between the experimentally meas-

urable quantities and the initial vacancy production mechanisms. For example, single and double ionization of helium are identically equal to single and double K vacancy formation. However, for heavier targets, various forms of outer- and inner-shell ionizations with subsequent relaxation modes can result in the same final target ionization charge states. Thus, by combining these various pieces of information, it is possible to separate and identify these individual cross sections. An example for double ionization of argon is shown in Figure 1, in which the total double ionization cross section ($\sigma^{\text{I}2}$) is broken into its charge transfer ($\sigma^{\text{I}0}_2$ and $\sigma^{\text{I}1}_2$) and direct ionization ($\sigma^{\text{I}1}_2$) components. These, in turn, are broken into their respective outer-shell (M) and inner-shell (L) contributions.

By separating and identifying the cross sections for single and multiple outer-shell ionization, we have been able to demonstrate that direct multiple outer-shell ionization occurs via a multistep projectile/target interaction for low to intermediate impact

Legend:

σ_2^T : ———, total double ionization cross section;
 σ_2^{11} : • and ▼, direct double ionization;
 σ_2^{10} : ○ and — · —, double ionization resulting from single electron capture by the proton;
 σ_2^{1-1} : — · · —, double electron capture by the proton;
 σ_m^{11} : · · · ·, direct double outer-shell ionization;
 σ_L^T : — — —, total L shell ionization cross section;
 σ_{LM}^T : — · · —, total L shell ionization resulting in double ionization of argon;
 σ_{LM}^{10} : — · · ·, L shell capture resulting in double ionization of argon.

FIGURE 1. Double Ionization of Argon Occurring as a Result of Proton Impact.

energies. For very high impact energies, a single-step interaction dominates; both types of interactions are important for a transition region of energies. Here, single-step and multistep interactions correspond to terms of a perturbation expansion that can be used to calculate these cross sections.

In addition, we have been able to demonstrate how the various Auger relaxation modes of inner-shell vacancies lead to varying degrees of target ionization. We have been able indirectly to quantify double Auger decay cross sections, which generally cannot be identified or measured for ion impact.

A publication describing these investigations, "A Study of Multiple Ionization Channels in Proton-Atom Collisions," has been accepted for publication in Physical Review A.

REFERENCES

- DuBois, R. D. 1984. "Electron Production in Collisions Between Light Ions and Rare Gases: The Importance of the Charge-Transfer and Direct-Ionization Channels." Phys. Rev. Lett. 52:2348.
- DuBois, R. D., L. H. Toburen, and M. E. Rudd. 1984. "Multiple Ionization of Rare Gases by H^+ and He^+ ." Phys. Rev. A 29:70.
- Rudd, M. E., R. D. DuBois, L. H. Toburen, C. A. Ratcliffe, and T. V. Goffe. 1983. "Cross Sections for Ionization of Gases by 5-4000 keV Protons and for Electron Capture by 5-150 keV Protons." Phys. Rev. A 28:3244.

TARGET IONIZATION BY HELIUM ION IMPACT

R. D. DuBois

An extensive data base providing information on detailed mechanisms that lead to ionization of atoms by proton impact has been developed. Thus we are now turning our attention to "clothed" ion impact; that is, to projectiles arriving at the collision region with bound electrons of their own. Our initial investigations have concentrated on He^+ impact in the study of both total ion and electron production (Rudd et al. 1985) and the amount of multiple ionization that occurs (DuBois, Toburen, and Rudd 1984). Additional details at lower impact energies have been obtained for the direct ionization (σ_q^{11}) and the electron capture (σ_q^{10}) channels (DuBois 1984). It was shown that the predominant electron production mechanism for slow He^+ impact can be the multiple ionization channel; namely, electron capture plus one or

more additional target ionizations. This was found to be even more important in the case of He^{2+} impact, where both the single and the double electron capture channels can contribute to the free electron production as effectively as does the direct ionization channel (DuBois 1986). Figure 1 shows that the single and double capture plus one additional target ionization cross sections (σ_{21}^{21} and σ_{20}^{20}) are comparable to the direct single ionization cross section (σ_{11}^{11}). Each of these channels is responsible for creating one free electron.

These measurements were originally made for impact energies lower than 100 keV/atomic mass unit (amu), but in the case of He^+ impact measurements have now been extended to 500 keV/amu. At these higher impact energies, the importance of the electron capture channel is rapidly decreasing; however, the electron loss channels (σ_{12}^{12}) become very important, as can be seen in Figure 2. Note that the total electron emission (σ^-) differs considerably from the direct single ionization cross section (σ_{11}^{11}) because multiple ionization plays an important role in all target ionization mechanisms. It has also been observed that electron loss from the projectile almost always results in simultaneous target ionization since $\Sigma \sigma_{12}^{12} \approx \sigma_{12}^{12}$, thus implying that σ_{12}^{12} is small.

Details as to how this simultaneous projectile/target ionization influences the electron emission spectra will be discussed in the following section.

REFERENCES

- DuBois, R. D. 1984. "Electron Production in Collisions Between Light Ions and Rare Gases: The Importance of the Charge-Transfer and Direct-Ionization Channels." Phys. Rev. Lett. 52:2348.
- DuBois, R. D. 1986. "Ionization and Charge Transfer in He^{2+} - Rare Gas Collisions." Phys. Rev. A 33:1595.
- DuBois, R. D., L. H. Toburen, and M. E. Rudd. 1984. "Multiple Ionization of Rare Gases by H^+ and He^+ Impact." Phys. Rev. A 29:70.
- Rudd, M. E., T. V. Goffe, A. and R. D. DuBois. 1985. "Cross Sections for Ionization of Gases by 10-2000 keV He^+ Ions and for Electron Capture and Loss by 5-350 keV He^+ Ions." Phys. Rev. A 32:829.
- Rudd, M. E., T. V. Goffe, and A. Itoh. 1985. "Ionization Cross Sections for 10-300 keV/amu $^3\text{He}^{2+}$ Ions in Gases." Phys. Rev. A 32:2128.

FIGURE 1. Cross Sections for Ionization of Argon by He^{2+} Impact. Total yield of target ions σ_+ : + present data, ---- Rudd, Goffe, and Itoh 1985; total yield of electrons σ_- : - present data, ---- Rudd, Goffe, and Itoh 1985; total single (---- Rudd, Goffe, and Itoh, 1985) and double (---- Rudd, Goffe, and Itoh 1985, x present data) electron capture cross sections. Cross sections for producing a q times ionized target ($q = 1-5$) resulting from direct ionization (σ_q^{22}), single (σ_q^{21}) and double (σ_q^{20}) charge transfer are as indicated.

FIGURE 2. Cross Sections for Ionization of Neon by He^+ Impact. Total yield of target ions σ_+ : + present data, — Rudd et al. 1985; total yield of electrons σ_- : - present data, — Rudd et al. 1985; direct single-, double-, and triple-target ionization σ_q^{11} ($q = 1, 2, 3$): ●, ■, ▲; total single electron capture cross section σ^{10} : ▽ present data, — Rudd et al. 1985; single-, double-, and triple-target ionization resulting from single capture collisions σ_q^{10} ($q = 1, 2, 3$): ●, ■, ▲; total electron loss cross section σ^{12} : — Rudd et al. 1985; single-, double-, and triple-target ionization resulting from electron loss collisions σ_q^{12} ($q = 1, 2, 3$): ●, ■, ▲.

STRUCTURED ION IMPACT: DOUBLY DIFFERENTIAL CROSS SECTIONS

R. D. DuBois

Electron emission occurring as a result of "clothed" ion impact (where the projectile contains electrons of its own) on atomic and molecular targets is difficult to model theoretically. Not only must the target ionization induced by a partially screened projectile nuclear charge be calculated, but projectile ionization induced by a neutral target also must be considered. In addition, it is possible that simultaneous ionization of the collision partners can occur. The doubly differential cross sections that we have traditionally measured in our laboratory are the sum of these individual electron production mechanisms. However, in a new series of experiments, we have measured the electron emission in coincidence with a projectile that has been ionized, thus making it possible to separate and identify electrons resulting from these various mechanisms.

In 1985, coincidence doubly differential cross sections were measured for 400 to

750 keV/atomic mass unit (amu) He^+ impact on He, Ne, Ar, Kr, and H_2O . Cross sections were measured for selected angles and for electron energies ranging from 10 to 1000 eV. A representative example of these data, recently published in *Physical Review Letters* (DuBois and Manson 1986), is shown in Figure 1. Because of the coincidence mode of measurement, the total electron emission (dotted curve) has been subdivided into its target emission (Δ) and its projectile emission ($\diamond$) components. The most interesting findings were that, although projectile emission is effectively responsible for the entire electron loss peak observed in the electron emission spectrum near 250 eV (as was expected), target ionization does not account for the total electron emission spectrum at lower electron energies (a totally unexpected discovery). A sizable percentage of these low-energy electrons were shown to originate as a result of simultaneous projectile/target ionizations (see, for example, the discussion in "Target Ionization by Helium Ion Impact" in this report). Similar features were observed for all targets and impact energies that were studied.

FIGURE 1. Doubly Differential Cross Sections for 500 keV/amu $\text{He}^+ - \text{He}$ Collisions. The laboratory electron emission range is 20° . Total electron emission:exp, _____ theory; target electron emission: $\blacktriangle$ exp, ---- theory; electron emission associated with projectile ionization; $\diamond$ exp, -.-.- theory.

Also included in Figure 1 are theoretical calculations made using the Born approximation. A comparison of the calculations with the experimental data shows that target ionization and pure projectile ionization are handled fairly well theoretically but that simultaneous projectile/target ionization is drastically underestimated. This comparison also provides information about charge transfer to the continuum, which is assumed to account for the discrepancy between the experimental and theoretical target ionization cross sections near 250 eV. Thus these data have already provided insights into future improvements of the theoretical procedure

used to calculate "clothed" ion impact ionization cross sections.

Future work is planned for H^0 impact, where it is anticipated that simultaneous projectile/target ionization will play a less significant role.

REFERENCE

DuBois, R. D. and S. T. Manson. 1986. "Coincidence Study of Doubly Differential Cross Sections: Projectile Ionization in $\text{He}^+ - \text{He}$ Collisions." *Phys. Rev. Lett.* 57:1130-1132.

DOUBLY DIFFERENTIAL IONIZATION CROSS SECTIONS FOR INTERMEDIATE-VELOCITY HEAVY IONS

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

The interaction of fast neutrons in tissue results in the production of recoil ions that deposit energy along densely ionizing charged particle tracks. Although a great deal of information exists regarding ionization by the recoiling of protons and by nuclear reaction induced alpha particles, little is known about the detailed ionization cross sections for the heavier recoil ions (such as carbon, oxygen, and nitrogen) that also may be produced by interactions of neutrons in tissue. In previous progress reports, we have discussed our measurements of differential ionization cross sections for carbon ions. During the past year we have extended these measurements to oxygen ions in water vapor targets.

Measurements were conducted for oxygen ion energies from 1.3 to 3.3 MeV. Cross sections differential in electron energy and emission angle were measured for angles ranging from 15° to 130° and for ejected electron energies from a few eV to nearly 1500 eV. Of particular interest are the effects of varying projectile charge state, ion energy, and target structure on the electron production cross sections.

The singly differential cross sections, $\sigma(\epsilon)$, obtained for three different projectile charge states of 3.28-MeV oxygen ions, are compared in Figure 1. As expected, the cross sections for emission of low-energy electrons increase as the ion charge state increases. For these distant collisions the nuclear charge of the projectile is effectively screened by its bound electrons; thus the interaction strength is proportional to its net charge. Note, however, that even for emission of electrons as low as 10 eV sufficiently soft collisions have not been reached for Z^2 scaling to be appropriate to projectile charge; the cross section for charge state three is only about a factor of two higher than those for charge state one. For high-energy ejected electrons, we expect

FIGURE 1. Effect of Variations in the Incident Ion Charge State for Singly Differential Electron Emission Cross Sections in Collisions of 3.28-MeV Oxygen Ions with Water Molecules

collisions at very small impact parameter, at which electronic screening by ionic electrons is minimal; thus, similar cross sections are expected for all charge states. The comparison in Figure 1, however, shows a clear decrease in the cross sections for electrons ejected with energies higher than about 500 eV by the higher-charged ions compared to electrons of similar energy that are ejected by lower-charged ions. The reason for this decrease in cross section for an increase in ion charge state is not apparent in this preliminary analysis.

For intermediate- to low-energy ion collisions, represented by the energy range under study, we may speculate that the emission cross sections should depend more on the kinetic parameters of the collision and the total number of electrons involved than on the detailed atomic or molecular structure of the colliding partners. This is deduced by considering the interaction to proceed through formation of a quasi-molecule with

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electron emission being induced via Pauli excitation as the bound orbitals of the independent systems interact. Because outer-shell ionization involves numerous potential curve crossings, we would expect rearrangement on a statistical basis, with individual states losing their identity relative to the observed electron spectra.

If this concept has any validity, we would expect similar electron spectra and yields to be produced by collision systems of similar relative velocities and total electron number. In Figure 2 we compare our recent measurements for 0.2 MeV/amu (3.2 MeV) O^+ ions on water vapor with our previous data for 0.2 MeV/amu (2.4 MeV) C^+ ions on neon. Note the similarity in the results. For high-energy ejected electrons the oxygen ion cross sections are larger than the carbon results, indicating the somewhat larger Z^2 scaling for these small impact parameter collisions, i.e., $Z_O^2/Z_C^2 \sim 2$, where Z is the nuclear

charge. For low-energy ejected electrons, large impact parameter collisions where bound electrons provide screening of the nuclear charge of the ion, the emission cross sections are nearly equal for the two ions.

Also shown in Figure 2 are results for O^+ and C^+ ions of very similar energy. These results are in even closer agreement than ions of equal velocity. This agreement may, however, be fortuitous as the increase in effective charge for oxygen relative to carbon ions for ejection of fast electrons may just counteract the shift of the oxygen ion spectrum to lower electron energies because of the slower velocity of that ion. These systematics as well as ion energy and target effects are under continued study.

BETHE-BORN THEORY OF IONIZATION BY HIGH-VELOCITY IONS AND ELECTRONS

J. H. Miller and W. E. Wilson

A comprehensive and accurate data base of collision cross sections is required for a detailed description of the absorption of ionizing radiation in biologically significant materials. Although experiments are the primary source of these data, theories of the interaction of radiation with atomic and molecular targets are also needed to fill gaps where experiments are particularly difficult and to test the consistency of related data. Bethe's asymptotic expansion of the first Born approximation (Inokuti 1971) has been particularly useful for this purpose because it achieves an approximate separation of the dependence of collision cross sections on projectile and target properties.

Two contributions on the application of Bethe theory to the analysis of differential ionization cross sections were made this year. The first of these involves the energy spectrum of secondary electrons that are ejected from water by fast bare ions and electrons. A paper on this topic has been accepted for publication in the *Journal of Chemical Physics*. In an earlier publication (Wilson et al. 1984), coefficients in the Bethe theory that describe the dependence of singly differential cross sections (SDCS) on the electronic structure of the water molecules were evaluated from optical and proton-impact ionization data and were used to predict the

FIGURE 2. Comparison of Single Differential Electron Emission Cross Sections for Ionization of Neon by C^+ Ions and Ionization of Water Molecules by O^+ . The upper curves compare ions of equal velocity; the lower curves compare ions of nearly equal energy.

dependence of SDCS on ion energy. Recently, more data on ionization of water by protons and electrons have become available (Rudd et al. 1985; Bolorizadeh and Rudd 1986), and we have incorporated these new data into a re-examination of the Bethe coefficients for water. The new coefficients permit more accurate calculations of SDCS for ejection of low-energy secondary electrons and make our model of the secondary-electron energy spectrum more consistent with total ionization cross sections and stopping power.

Our second contribution in this area concerns the spectrum of secondary electrons produced when structured ions collide with atomic and molecular targets. The presence of electrons bound to the projectile causes the secondary-electron spectrum to contain contributions from both projectile stripping and target ionization. These components must be separated in order to use the data in track structure simulations. Because measurements of the emission characteristics of secondary electrons are not usually made in coincidence with measurements of projectile charge state, theoretical work is needed to separate the components of the secondary-electron spectrum and to extend the range of existing measurements. Models for this purpose were presented at the thirty-fourth annual meeting of the Radiation Research Society in Las Vegas.

As was the case for bare ions, these models of ionization by structured ions rely on the separation of target and projectile properties afforded by the first Born approximation to the quantum collision theory. The Compton profile of the projectile gives the contribution to the momentum of electrons moving with the projectile from their motion about the nucleus of the projectile. Given the total momentum of these electrons, stripping can be viewed as electron scattering by the target (Jakubassa 1980). The dominant contribution to the differential stripping cross section is provided by elastic scattering, but inelastic scattering may also occur; this phenomenon contributes to the cross section for simultaneous projectile stripping and target ionization.

The dominant role of bound electrons on the projectile in target ionization is screening of the projectile's nuclear charge. Because high-energy secondary electrons tend to be ejected in collisions with small impact parameter, the effectiveness of this screening is strongly correlated with secondary-electron energy. However, the relationship between projectile screening and the energy of ejected electrons can be included in the Bethe coefficients, which are evaluated from photoionization data and cross sections for ionization of the target by the structured ion at a single ion energy. These modified Bethe coefficients are no longer simply a function of the target's electronic structure since they also depend on the electronic state of the structured ion. Nevertheless, they are independent of the velocity of the projectile and hence can be used to predict SDCS as a function of ion energy.

REFERENCES

- Bolorizadeh, M. A., and M. E. Rudd. 1986. "Angular and Energy Dependence of Cross Sections for Ejection of Electrons from Water Vapor. I. 50-2000-eV Electron Impact." Phys. Rev. A 33:882-896.
- Inokuti, M. 1971. "Inelastic Collisions of Fast Charged Particles with Atoms and Molecules--the Bethe Theory Revisited." Rev. Mod. Phys. 43:297-347.
- Jakubassa, D. H. 1980. "Electron Loss from Medium-Energy Projectiles in Collisions with Heavy Target Atoms." J. Phys. B 13:2099-2108.
- Rudd, M. E., T. V. Goffe, R. D. DuBois, and L. H. Toburen. 1985. "Cross Sections for Ionization of Water Vapor by 7-4000-keV Protons." Phys. Rev. A 33:492-494.
- Wilson, W. E., J. H. Miller, L. H. Toburen, and S. T. Manson. 1984. "Differential Cross Sections for Ionization of Methane, Ammonia, and Water Vapor by High Velocity Ions." J. Chem. Phys. 80:5631-5638.

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 Institute 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.

APPLICATION OF TRACK STRUCTURE IN MICRODOSIMETRY

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

A paper on proton ionization and energy deposition in submicron sites, entitled "Microdosimetric Aspects of 0.3 to 20 MeV Proton Tracks: I. Crossers" has been prepared for submission to Radiation Research; the abstract is presented here.

Comprehensive results are presented for the evaluation of microdosimetric quantities from track structures of fast protons obtained by computer simulation using a detailed-history Monte Carlo method. Stochastic frequency distributions for energy imparted and number of ionizations 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 these the dependencies of the first two moments of the distributions on ion energy, site size, and position of the track within the site were derived. The frequency distributions are fitted in the least-squares sense by simple analytic (lognormal) functions to facilitate their use in further microdosimetric considerations.

AN ANALYTIC MODEL FOR IONIZATION DISTRIBUTIONS PRODUCED IN NANOMETER SITES BY PROTONS

W. E. Wilson and H. G. Paretzke

Extensive computer simulations of proton tracks have been made for protons of energy E , passing through spherical sites of diameter d , off center by a distance b , which we call eccentricity. Computed ionization distributions for $0.3 < E < 20$ MeV, $2 < d < 1000$ nm, and $0 < b < d/2$ were obtained. The lognormal function,

$$f(j) = \frac{\text{EXP}(-(\ln(j) - \mu)^2 / 2\sigma^2)}{\sigma j (2\pi)} \quad (1)$$

is used to represent these distributions phenomenologically. To achieve this representation the function was fitted in the least-squares sense to the ionization distributions, thus providing the dependence of the two parameters μ and σ on E , d , and b . The dependence of μ on E and d is rather simple; μ can be described mathematically by the expression

$$\mu = c_1 \log_{10}(d) + c_2 \log_{10}(E) + c_3(b) \quad (2)$$

where c_1 and c_2 are independent of eccentricity and c_3 is independent of both E and d . The values of $c_1 = 2.55817$ and $c_2 = -2.06716$ were obtained by multiple-linear regression of equation (2) to the μ versus E versus d data, and c_3 is given by

$$c_3 = 10. (0.45 - 0.5056 (1-b^2)^{1/2}) \quad (3)$$

The width parameter σ is obtained from the ratio of the variance, V , to the mean, $\langle j \rangle$, of the distributions in ionization. This ratio is essentially independent of ion energy E up to a given diameter, at which point the ratio saturates. The size of this diameter depends on ion energy because, as the diameter is increased, the site eventually becomes large enough to contain the most energetic delta-ray for that ion energy; i.e., all deltas become stoppers.

We have approximated the behavior of the ratio, $V/\langle j \rangle$, by

$$(V/\langle j \rangle) = d^{0.556} \quad (4)$$

and the saturation value by

$$(V/\langle j \rangle_{\max}) = 1385. E/\ln(69.1 E) \quad (5)$$

It can be shown that, for the lognormal distribution,

$$\langle j \rangle = \exp(\mu + \sigma^2/2) \quad (6)$$

and

$$V = (\exp(\sigma^2) - 1) \exp(2\mu + \sigma^2) \quad (7)$$

These two equations can be combined and reduced to

$$g(\sigma^2) = \sigma^2/2 + \ln(\exp(\sigma^2) - 1) + \mu - \ln(R) = 0 \quad (8)$$

where $R = V/\langle j \rangle$.

The width parameter σ is obtained by numerically solving for the zero of equation (8). A concise computer code system written in FORTRAN is under development to evaluate this model of the stochastics of ionization.

For high-velocity charged particles and sufficiently small sites it is quite possible for the particle to pass through the site without depositing any energy or without ionization. This situation indicates a breakdown in the proportionality between fluence and dose, and it is therefore desirable to know how frequently this happens and, ideally, to be able to quantify the frequency. From the computer simulations it was discovered that in sites smaller than 1000 nm in diameter, the frequency for zero ionization is simply related to the cross section for ionization,

$$f(j=0) = \exp \left[- \frac{d (1 - b^2)^{1/2}}{\lambda_j(E)_p} \right] \quad (9)$$

where j is the ionization number and λ_j is the mean free path for the interaction. The numerator of the exponential argument in equation (9) is just the path length of the ion through the site when it has a relative eccentricity of b . Equation (9) gives an upper limit for the frequency for zero interaction, because ionization produced by secondary electrons should sometimes be a contributing factor and thereby reduce the frequency for zero interaction. That this does happen has been confirmed by the simulations.

MICROSCOPIC DOSIMETRY OF IONS IN NANOMETER SITES: TOUCHERS

W. E. Wilson and H. G. Paretzke

Two types of events are of primary importance in the submicron dosimetry of fast protons. The foremost are those events caused by protons that pass through the site, and therefore called "crossers." Second are events caused by protons that pass outside the site but that deposit energy via secondary electrons (delta-rays) that do enter the site. Such events have been called "touchers" in the literature.

As part of our continuing study of the systematics of proton track structure, we are computing the ionization and energy deposition distributions for toucher events for 0.3 to 20 MeV protons and sites from 10 to 200 nm as a function of radial distance (eccentricity) from the proton path. Results to date,

since this type of event is rare, are best described by the radial dependence of the total event frequency (i.e., one minus the frequency for zeros), and by the frequency distribution for event size, Y , when a non-zero event does occur.

The computations indicate that the probability for a nanometer site experiencing any ionization deposition decreases very rapidly with eccentricity and the functional dependence on eccentricity appears to approach $1/r^2$ asymptotically for high-velocity ions. This behavior is exhibited in Figure 1 for 10 MeV protons passing near 50, 100, and 200-nm-diameter sites. Furthermore, the results indicate that at large eccentricity the probability of an event is proportional to the square of the site diameter, i.e., to the solid angle of the site as seen from the ion path, and proportional to $\log(E)/E$, i.e., to the total ionization cross section for the ion of energy E . In other words, the probability is proportional to the number of delta-rays produced per unit path length.

At small eccentricity the frequency for an event drops more rapidly than $1/r^2$. This is consistent with the behavior of a line source of radiation in an absorbing medium

(see, for example, Morgan and Turner 1973). The actual shape of the radial dependence of the event frequency is not predictable by any simple function. For monoenergetic radiations, having a single absorption coefficient, the shape is given by the Sievert integral (Morgan and Turner 1973). But here, that is not the case and the shape probably arises out of the singly differential cross section (SDCS) for the production of delta-rays and their energy-dependent absorption characteristics. Because no simple theoretical formulation is readily available, we expect to use a phenomenological representation.

At large eccentricity, the frequency distribution in event size does not vary significantly with eccentricity, as indicated by the relatively constant mean event size shown in Figure 2. At smaller eccentricity the mean event size is smaller, presumably reflecting the effect of the very numerous subionization delta-rays produced along the ion path. This agrees with actual measurements on ion beams (Glass and Roesch 1972; Gross and Rodgers 1972). It is also consistent with what we would expect for the event-size distribution for intermediate-energy delta-ray crossers, which are presumably dominating.

FIGURE 1. Total Delta-Ray-Event Frequency as a Function of Radial Distance from the Proton Path. The frequency depends approximately on the distance squared. The frequency goes to zero at larger distances as the effective maximum delta-ray range is approached.

FIGURE 2. Mean Event Size as a Function of Radial Distance from the Proton Path. At these large distances the event size is rather constant. At smaller eccentricity the event size is much smaller, probably because of the numerous subionization delta-rays, which have very short ranges.

Toucher-event modeling will focus on discovering the systematics of the distributions and describing them with simple analytic functions, thereby completing the description of bare-proton track structure up to 20 MeV.

REFERENCES

- Glass, W. A., and W. C. Roesch. 1972. "Measurement of Ionization Distributions in Tissue-Equivalent Gas." Radiat. Res. 49:477-494.
- Gross, W., and R. C. Rodgers. 1972. "Heavy-Ion Event Spectra." In Proceedings of the Third Symposium on Microdosimetry, vol. 3, pp 873-887. Ed. H. G. Ebert. October 18-22, 1971, Stresa, Italy. Commission of European Communities, Luxembourg.
- Morgan, K. Z., and J. E. Turner, eds. 1973. Principles of Radiation Protection. Robert E. Kriegler Publishing Co., Huntington, New York, p. 277 ff.

TRACK STRUCTURE FOR 10- TO 300-keV PROTONS

W. E. Wilson

Simulation of ion track structures has been extended down to an ion energy of 10 keV by developing algorithms for the charge-exchange cross sections (Toburen, Nakai, and Langley 1967, 1968) and the accompanying delta-ray spectra. These results must be considered preliminary, however, because of the approximate nature of some of the code extensions. For the single differential cross section (SDCS) for delta-ray emission, we have made use of a "hydrogenic approximation" by Professor M. E. Rudd. The Rudd algorithm is for low-energy ion-induced delta-ray emission from hydrogen rather than water. We use it to estimate emission from each valence shell of water vapor by allowing for the different binding energies, and then sum the shells to get a total for the eight valence electrons in the molecule. This procedure produces an ion stopping power that is about 25% too large around 50 to 100 keV, decreasing on each side of this to become 5% to 10% too small at 10 and 300 keV.

Calculations of distributions in energy imparted and also ionization number have been made for 10- to 200-keV protons in sites from 2 to 100 nm in diameter. These preliminary results indicate that the distributions are more symmetric than those produced by higher-energy ions. This is to be expected because the ionization cross sections are larger, at least above 50 keV. Hence more interactions occur on the average within a given site size than occur for higher-energy ions; larger mean values produce more symmetric Gaussian-like distributions.

Future improvements in ion track structure simulation will include the possibility for multiple ionization of the target molecule. However, this extension awaits more comprehensive cross-section measurements.

REFERENCES

- Toburen, L. H., M. Y. Nakai, and R. A. Langley. 1967. "The Measurement of High-Energy Charge Transfer Cross Sections for Incident Protons and Atomic Hydrogen in Various Gases and the K-, L-, and M-Auger, L-Coster-Kronig, and the Conversion-Electron Spectra of Platinum in the Decay of ^{195}Au ." Thesis, ORNL-TM-1988, Oak Ridge National Laboratory, Oak Ridge, Tennessee.
- Toburen, L. H., M. Y. Nakai, and R. A. Langley. 1968. Phys. Rev. 171:114-122.

MICROSCOPIC TRACK STRUCTURE OF EQUAL LINEAR-ENERGY-TRANSFER HEAVY IONS

W. E. Wilson and T. L. Criswell*

The spatial distributions of ionization and energy deposition produced by heavy (HZE) ions are crucial to an understanding of their radiation quality as exhibited, for example, in track segment experiments of cell survival and chromosome aberrations of mammalian cells. The stopping power, or linear energy transfer (LET), of a high-velocity ion is proportional to the ratio Z^2/v^2 , where Z and v are the atomic number and velocity, respectively, of the moving ion, apart from a

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slowly varying logarithmic factor. The maximum delta-ray energy that an ion can produce is proportional to v^2 (nonrelativistically). Therefore, two HZE ions having the same LET, but in general different Z and v , will have different maximum delta-ray energies and consequently will produce different spatial patterns of energy deposition along their paths. To begin to explore the implications of this fact for the microscopic dosimetry of heavy ions, we have calculated radial distributions in energy imparted and ionization for iron and neon ions of approximately equal LET to directly compare their delta-ray track structure. Monte Carlo techniques are used for the charged-particle radiation transport simulation. Results of these preliminary studies were reported in a paper presented at the XXVI Plenary Meeting of the Committee on Space Research (COSPAR), June 1986, Toulouse, France.

RADICAL YIELDS IN ORIENTED DNA EXPOSED TO HIGH LINEAR-ENERGY-TRANSFER RADIATION

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We are developing track structure-based models to calculate the yield of free radical species in oriented DNA fibers exposed to ionizing radiation with large LET. Experiments performed at AFRRRI show an unusual effect: radical yields detected by electron spin resonance (ESR) at 77°K depend on the relative orientation of the axis of the DNA molecules to the flux of ionizing particles. When a neutron flux is incident perpendicular to the DNA, ESR spectra are consistent with the production of roughly equal amounts of thymine anion and guanine cation. However, when the sample is irradiated with the neutron flux approximately parallel to the DNA axis, yields of thymine anion and guanine cation are reduced and a new ESR signal, characteristic of the 5,6-dihydro-5-thymyl (TH) radical, is seen.

The amount of energy absorbed in DNA relative to that absorbed in the aqueous matrix of the fibers is primarily determined by their respective volume and is independent of orien-

tation. Consequently, the orientation dependence of radical yields must be related to the spatial distribution of energy deposition events rather than to the total amount of energy absorbed in DNA. The most obvious difference in the pattern of energy deposition between the two irradiation geometries is that charged particles that traverse the sample nearly parallel to the helical axis of DNA have a greater probability of multiple-energy transfer to the same molecule than do particles moving perpendicular to this axis. However, this difference in the spatial distribution of energy absorption should have no consequence for radical production unless intramolecular energy transfer greatly exceeds intermolecular energy transfer. Hence, it is the implication for intramolecular energy transfer that makes the unusual orientation dependence of radical yields important for radiation biology.

Although singlet and triplet excitations appear to be "self trapped" (migration of more than 10 base pairs is highly improbable), the presence of a conduction band in nucleic acids and the transport of vibrational excitation are still open questions. Enhanced recombination due to intramolecular charge transport may account for a part of the reduction in the yield of thymine anion and guanine cation; however, we postulate that the appearance of TH radicals in the parallel irradiation geometry is due to intramolecular vibrational energy transport. This hypothesis is consistent with the findings of Gräslund et al. (1975), who investigated the formation of TH by annealing of oriented DNA that was gamma irradiated at 77°K. From the analysis of their kinetic data, Gräslund et al. conclude the following: (i) TH is produced by thermally activated proton transfer to thymine anion, (ii) water of hydration is the most likely proton source, and (iii) the reaction has a distribution of activation energies between 0.2 and 0.7 eV.

The possibility of radiation-induced thermal activation has been recognized for some time (Mozumder 1969). The excess temperature induced by the isolated energy deposition events that are characteristic of sparsely ionizing radiation dissipates too rapidly to effectively induce chemical reactions; however this may not be true for high-LET radiation. Assuming a large asymmetry in thermal

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conductivity allows rapid transport of vibrational energy along a DNA molecule but not between molecules, our model predicts the following result for the yield of TH relative to the yield of thymine anion:

$$\frac{g_{TH}}{g_T} = \frac{\sum_i \epsilon_i \int_0^{t_{\max}} k(X_i, t) dt \exp\left[-\int_0^t k(X_i, s) ds\right]}{\sum_i \epsilon_i} \quad (1)$$

where

$$K(X_i, t) = A \exp[-Q/T(X_i, t)] \quad (2)$$

with

$$T(X_i, t) = T_s + \sum_{k+i} T_k \left(1 + \frac{2Dt}{\Delta^2}\right)^{-1/2} \exp\left[\frac{(-X_1 - X_k)^2}{(2\Delta^2 + 4Dt)}\right] \quad (3)$$

In these equations, x denotes the position of an energy deposition event and ϵ is the amount of energy absorbed by DNA in that event. These stochastic variables are averaged in our model of the experimentally observed radical yields. The expression for the proton transfer reaction rate, $k(x, t)$, is characterized by a rate constant A that is on the order of vibration rates ($10^{12}/s$) and the activation energy Q discussed above. The temperature at the position of a thymine anion, $T(x_i, t)$, is the sum of the sample temperature and the contribution from other energy transfers to the molecule from the slowing down of a given charged particle. The contribution of an energy absorption at position x_i to the excess temperature at x_k depends on initial excess temperature T_k (proportional to ϵ_k), the distance between x_i and x_k , and the migration of energy along the DNA molecule in the time interval t . In this model, the migration of energy is characterized by its initial spread Δ due to subpicosecond processes and the thermal diffusion coefficient D . Although not known precisely, it is reasonable to assume that Δ is on the

order of the base-pair stacking distance (3.4 Å) and D is comparable to that of water ($10^{-3} \text{ cm}^2/s$).

Figure 1 shows results obtained from Equation (1) for a flux of 1-MeV protons incident either parallel or perpendicular to the helical axis of DNA. For activation energies in the neighborhood of 0.25 eV, the yield of TH per thymine anion is significantly greater in the parallel irradiation geometry. Averaged over the distribution of activation energies proposed by Gräslund et al. (1975), our model predicts that the yield of TH in the parallel irradiation geometry will be about a factor of 10 larger than the yield when DNA is irradiated by a flux of 1-MeV protons that is perpendicular to its helical axis. Although this result is in qualitative agreement with the experiments performed at AFRRRI, more calculations are required before a quantitative comparison can be made. In particular we must consider the variations in LET and angular divergence of recoil protons generated by neutron irradiation. These calculations are in progress.

FIGURE 1. Yield of 5,6-dihydro-5-thymyl Radical per Thymine Anion Precursor for 1-MeV Protons Incident on a Sample of DNA Oriented Either Perpendicular or Parallel to the Helical Axis

REFERENCES

- Gräslund, A., A. Ehrenberg, A. Rupperecht, B. Tjallden, and G. Strom. 1975. "ESR Kinetics of the Free Radical Conversion in γ -Irradiated Oriented DNA." Radiat. Res. 61:488-503.
- Mozumder, A. 1969. "Charged Particle Tracks." In Advances in Radiation Chemistry 1:1-102. Ed. M. Burton and J. L. Magee. Wiley-Interscience, New York.

• 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 being 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 homogeneous track models, have also been carried out.

A COMPARISON OF MICROSCOPIC DOSE WITH AVERAGE DOSE NEAR HIGH-ENERGY IRON IONS

N. F. Metting and L. A. Braby

Biophysical studies indicate that the mechanisms responsible for radiation effects in biological systems involve injury to cell substructures much smaller than a cell nucleus. To understand the molecular mechanisms responsible, it is important to know the probable amount of dose deposited in the microscopic volumes of interest for a particular biological end point. This probability depends on the type of radiation, and is expected to correlate with the biological effect observed.

At the Bevalac accelerator at Lawrence Berkeley Laboratory, a small proportional counter (PC) mounted in a large vacuum chamber was used to measure single-event energy deposition distributions as a function of the distance radially outward from the primary ion path, for a 1.3- μm -diameter site. The results of these measurements have been reported previously (Metting and Braby 1986) and were recently compiled in a technical report (Metting et al. 1986). Those results have now been analyzed in terms of the single-event specific energy z_F (microscopic dose) actually received by small volumes, and compared to the dose which is frequently calculated based on homogeneous track models.

The case of heavy ion passage through tissue is interesting because the local pattern of

energy deposition in critical volumes is strongly influenced by the frequency and range of secondary electrons. For example, there are volumes that are traversed by the primary ion itself, those that are crossed only by secondary electrons, and those that receive no energy deposition. Single-event energy spectra for 600 MeV/atomic mass unit (amu) iron ions were measured for each radial distance b , and the mean value $\bar{z}_F$ was calculated for each b . The upper set of rectangles in Figure 1 shows these $\bar{z}_F$ values graphed as a function of radial distance. Due to the poor statistics at large radial distances, the contents of successively wider ranges of b were summed for ease of analysis. The horizontal width of each rectangle takes into account this range of the radial distance. The vertical width takes into account one standard deviation based on the number of events detected, and assumes that the associated energies are Poisson-distributed. Since our measurements were done using a 1.3- μm -diameter volume, these values of $\bar{z}_F$ represent the "microscopic dose" that a volume of that size would receive if it were traversed by delta-rays originating at the specified radial distance. There is a steady decrease for about 5 μm radially outward, after which these values remain fairly constant for the distance measured. The initial decrease in $\bar{z}_F$ reflects the decreasing probability of encountering multiple numbers of secondary electrons crossing the site, and those sites which are crossed receive roughly the same distribution of energies, independent of the distance from the track.

FIGURE 1. Microdosimetric $\bar{z}_F$ Graphed as a Function of Radial Distance b Around 600 MeV/amu Iron, and Compared to "Local Dose" Values Calculated with a Homogeneous Track Structure Model

One homogeneous track structure model developed by Chatterjee, Maccabee, and Tobias (1973) predicts a quantity, Q_p called an energy density or "local dose," which is a function of the radial distance. The solid line in Figure 1 shows this quantity calculated from the model for 600 MeV/amu iron ions. Where the radial distance b is greater than the simulated site radius, the values of $\bar{z}_F$ are progressively larger than Q_p . This is as expected, since Q_p averages in all of the volume encompassed by the radial distance bins, i.e., sites with no energy deposition (zero events), are included.

The results obtained in our measurements can be compared to values calculated via Chatterjee's model by including the zero events and recalculating an average energy absorbed as a function of b . This is shown in Figure 1,

where values of $\bar{z}_F$ were multiplied by the event frequency per primary, for each radial distance. The resulting values are in good agreement with the local dose curve of Chatterjee.

It should be possible to use the results of measurements of one ion (iron) at one energy (600 MeV/amu) to extrapolate to other fully stripped ions for the same velocity. From stopping power theory, linear energy transfer (LET) is proportional to Z^2 , and we know from experiment that the shape of the secondary electron energy distribution depends primarily on the ion velocity. Thus, any bare ion species having a kinetic energy of 600 MeV/amu is necessarily traveling at a speed of $\beta = 0.8$, and will produce essentially the same secondary electron energy deposition spectrum. However, the number of secondaries will vary as Z^2 .

If one were to vary the velocity of the ion, the new delta-ray energy distribution would be predictable, but the deposition of energy in matter is a complex function of that distribution. Therefore, one cannot extrapolate precisely to determine $f_1(z)$ spectra for other velocities using only the measurements taken at one velocity. The best that can be done short of a Monte Carlo simulation (or direct measurement) is estimation via a Chatterjee type of track structure model.

REFERENCES

- Chatterjee, A., and H. J. Schaefer. 1976. "Microdosimetric Structure of Heavy Ion Tracks in Tissue." *Radiat. Environ. Biophys.* 13:215.
- Chatterjee, A., H. D. Maccabee, and C. A. Tobias. 1973. "Radial Cutoff Dose Calculations for Heavy Charged Particles in Water." *Radiat. Res.* 54:479-494.
- Metting, N. F., and L. A. Braby. 1986. "Experimentally Derived Values of $\bar{y}_D$ and $\bar{y}_D$ for 33.6 GeV Iron as a Function of Eccentricity." In *Physical Sciences. Part 4 of Pacific Northwest Laboratory Annual Report for 1985 to the DOE Office of Energy Research*. PNL-5750 PT4, Pacific Northwest Laboratory, Richland, Washington, pp. 29-31.

Metting, N. F., L. A. Braby, H. H. Rossi,
P. J. Kliauga, J. H. Schimmerling, M. Wong,
and R. Rapkin. 1986. Measurement of Energy

Deposition Near High Energy, Heavy Ion
Tracks. PNL-5950, Pacific Northwest Labora-
tory, Richland, Washington.

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. Models that are found to be consistent with data can be used in risk evaluation in the range of dose and dose rate where they have been tested, but their primary use comes in developing an understanding of the chemical and biological processes involved. Thus, different models that are characteristic of specific biochemical mechanisms or groups of mechanisms are contrasted to determine whether they result in different consequences at the cellular level. Often models based on quite different assumptions about mechanisms result in equivalent dose-effect relationships, but differences may 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 because more can be learned about specific mechanisms and their interactions by studying one system in detail than by cataloging the cell lines that possess a specific characteristic.

In some cases, important differences among models result in only small differences in cell survival within the ranges of dose and dose rate that are normally investigated. To overcome this limitation, new ways of irradiating cells at extremes of dose rate, or ways of evaluating the effects of very small doses, are developed. Mathematical modeling and cellular studies complement each other. New experimental techniques in molecular biology 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 interaction of dose and repair time as they affect 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 contribute to this effect.

CHARACTERISTICS OF REPAIR FOLLOWING VERY LOW DOSES

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

Repair of radiation-induced cellular damage plays a central role in the survival of cells exposed to doses of 1 Gy or more. This repair is responsible for the dose rate, split-dose and delayed plating effect and can be evaluated in the corresponding experiment. Because split-dose and dose-rate experiments involve repair during irradiation and delayed plating experiments involve repair after irradiation is completed, it was originally thought that different repair processes (i.e., removing sublethal and potentially lethal damage, respectively) were involved. It is now clear that this is not necessarily the case (Curtis 1985). Appropriately designed models can account for observed

effects at conventional doses (1 Gy or more) whether they assume all damage is lethal unless repaired or some damage is innocuous unless it interacts with additional damage. However, at low doses, where the probability of interaction between damage produced by separate charged particle events is very small, these two types of models predict significantly different responses. Both models predict that the interaction of products, in proportion to the square of their concentration, could produce the shoulder of the survival curve. Their predictions differ, however, when this interaction becomes rare. If the damage were potentially lethal, there would be a large delayed plating effect due to repair of the damage; however, if part of the damage were sublethal, the effect of delayed plating, which affects only part of the damage, would stay relatively constant.

We have utilized starved plateau-phase CHO cells to investigate this behavior. These cells are not cycling, so their radiation sensitivity is not changing with time. Consequently, 24-hour split-dose and delayed-plating intervals can be used to provide adequate time for even slow repair processes. The use of a fully automated clone counting system using a digitized video image of the assay plate enabled the counting of several thousand assay plates. At the lowest doses, 200 plates were typically used per point. Delayed-plating and split-dose effects were compared in one experiment, while others concentrated on improved resolution for delayed plating at low doses or following a low-dose-rate irradiation.

At doses above 1 Gy, repair intervals have the expected effect; i.e., they increase the survival. However, at lower doses the survival is decreased by both split-dose and delayed-plating schedules (Figure 1). This is accompanied by a prompt plating survival that is consistently higher than the control. The cause of the high survival is of some interest since it interferes with precise determination of the shape of the survival curve. Preliminary investigations have revealed that at 0.25 Gy the number of surviving clones is essentially equal to the number of cells placed in the assay dish, while the unirradiated controls show a plating deficiency of about 80%. The electronic particle counter used in this experiment is routinely calibrated against a hemocytometer, and there is no difference in the size distribution of irradiated and unirradiated

FIGURE 1. Comparison of Survival Following Immediate and Delayed Plating of Plateau-Phase CHO Cells Exposed to 250-kv X-Rays

cells as measured with a flow cytometer. It may be that those cells in the initial population that would not grow when replated are further damaged by a low dose of x-rays, and are made so fragile that they lyse during trypsinization. Thus the cells responsible for a plating efficiency of less than 1 would be removed from the irradiated population but not from the control group.

In any case, the fact that the survival following a plating delay is always less than the survival following immediate plating at low doses indicates that the damage produced is probably not potentially lethal. It may even be that repair plays a relatively minor role in modifying cell survival unless a relatively large amount of damage is present. Experiments with repair inhibitors are being undertaken to try to resolve this problem.

REFERENCES

Curtis, S. B. 1986. "Lethal and Potentially Lethal Lesions Induced by Radiation--A Unified Repair Model." *Radiat. Res.* 106:252-270.

ROLE OF REPAIR SATURATION IN THE RESPONSE OF PLATEAU-PHASE CHINESE HAMSTER OVARY CELLS

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

As reported previously (Nelson et al. 1985), we see two repair rates in split-dose experiments on starved plateau-phase CHO cells. We have assumed that this indicates two different processes repairing two distinct types of sublethal damage. However, as we reported last year (Nelson et al. 1986), results of experiments at different dose levels are not consistent with models that assume that the damage is entirely sublethal. Alternative hypotheses are being investigated to determine whether they provide a satisfactory interpretation of the data and a basis for further investigation of the repair mechanisms.

One hypothesis that has been considered is the saturation of a repair mechanism having a limited pool of repair enzymes. Such saturation phenomena have been observed in biochemical repair studies and have thus formed the

basis for a model of cellular response, which was shown to be capable of producing dose response curves in good agreement with experimental observations (Goodhead 1985). This model can be extended to account for both dose-rate and split-dose effects. In order to determine whether the observed response of CHO cells in split-dose experiments might be a result of the saturation of one repair process, we have analyzed this model in more detail.

In the model developed by Goodhead, as well as in most other repair saturation models, the damage produced by the interaction of radiation is assumed to be lethal unless repaired. The saturation of the repair process is then directly responsible for the shoulder of the survival curve, but it is not responsible for split-dose or dose-rate effects. These are produced by the replenishment of the (partially) depleted repair pool. Thus the two rates observed in a split-dose experiment would represent two separate repair systems that were being replenished at different rates. If the more rapidly replenished system did not saturate, the more slowly replenished one would not be observed. Unrepaired lesions are assumed to be the cause of the reproductive death of cells. If second rate can be detected in a split-dose experiment, the maximum number of effective lesions that can be repaired by the rapidly replenished process can then be determined. This number should be independent of the dose, reflecting only the size of the repair pool.

Data for typical equal-fraction split-dose experiments at 2 and 4 Gy per fraction are shown in Figure 1. At 4 Gy per fraction the amount of damage repaired by the rapidly replenished process is approximately twice the amount repaired by that process when the dose was 2 Gy. Thus we conclude that saturation of one repair process is not the cause of the two-component split-dose response in these cells. However, we had previously shown that an interaction of products in proportion to the square of their concentration was not the sole cause either. It may be that there are actually two or more repair processes contributing to the faster of the two rates observed in these split-dose experiments. A combination of a sublethal

FIGURE 1. Survival of Plateau-Phase CHO Cells After Split-Dose Irradiations Totalling 4 and 8 Gy

product being removed and a potentially lethal damage repair system being replenished might account for the dose dependence and extent of repair observed.

Another possibility is that the accessibility of damage to repair enzymes might be the limiting factor. In this case, the fast component of the split-dose data would reflect the rate of repair and the slow component would reflect the rate at which initially inaccessible damage becomes accessible to repair enzymes. A dose-effect model based on repair accessibility will be extended to predict the effects of split-dose irradiations, and alternative ways to test these alternative hypotheses of repair will be explored.

REFERENCES

- Goodhead, D. T. 1985. "Saturable Repair Models of Radiation Action in Mammalian Cells." *Radiat. Res.* 104:S-58-S-67.
- Nelson, J. M., N. F. Metting, L. A. Braby, and W. C. Roesch. 1985. "Sublethal Damage is Not the Same as Potentially Lethal Damage." In *Physical Sciences. Part 4 of Pacific Northwest Laboratory Annual Report for 1984 to DOE Office of Energy Research*. PNL-5500 PT4, Pacific Northwest Laboratory, Richland, Washington, pp. 37-39.

Nelson, J. M., L. A. Braby, and W. C. Roesch. 1986. "Distinguishing Alternative Accumulation Models." In *Physical Sciences. Part 4 of Pacific Northwest Laboratory Annual Report for 1985 to DOE Office of Energy Research*. PNL-5750 PT4, Pacific Northwest Laboratory, Richland, Washington, pp. 40-41.

REPAIR STUDIES WITH *CHLAMYDOMONAS REINHARDI* AND HIGH-LINEAR ENERGY TRANSFER RADIATIONS

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We have continued the use of high linear energy transfer (LET) to study the effect of track structure on the rate of repair in

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Chlamydomonas. The large uncertainties in the data obtained during Dr. Jacobson's 1985 visit were traced primarily to an unexpected effect of scattered beam on the dose calibration. We have now collected data on the dose-effect relationship and repair rate for electron, proton, and deuteron irradiations. We have also explored the use of 5-fluorodeoxyuridine (FUDR) inhibition of chloroplast deoxyribonucleic acid (DNA) production to reduce the redundancy in this extranuclear genome and to determine its effect on cell survival and repair kinetics. Although growth for a few days in 1.5×10^{-5} M FUDR drastically reduces the chloroplast DNA and results in readily detectable chloroplast mutations (Palmer 1985), we find no significant effect on radiation sensitivity or repair (Figure 1). Thus it appears that the chloroplast DNA of FUDR-treated cells is such

FIGURE 1. Survival of *Chlamydomonas* Grown in Normal and FUDR Media as a Function of Dose and Time Between Fractions in a Split-Dose Experiment with Electron Irradiation

a small fraction of the sensitive material within the cell that we will not be able to use it in kinetic studies with survival as the endpoint.

The repair of damage produced by fast ions apparently involves a complex mixture of processes. As reported earlier (Braby and Nelson 1985), the repair of hydrogen and helium ion-induced damage at relatively high surviving fractions is very similar to that of electron-induced damage. Repair times are essentially independent of LET, but there is an increase in irreparable damage with increasing LET. However, at larger doses, at which survival is below 10%, we find a different response beginning to dominate, as shown in Figure 2--a significantly slower process, resulting in a nearly constant rate of damage removal between 0 and 60 minutes. Due to the slow rate of this process and the difficulty of holding cells in a partial vacuum for extended times, we have not yet been able to determine the maximum extent of this repair. It appears that a relatively slow repair of an additional type of damage may be involved.

In this case the natural synchrony of *Chlamydomonas* is a disadvantage, since it prevents use of delayed plating experiments. However, repair-deficient mutants, such as those being investigated by G. D. Small at the University of South Dakota (Cox and Small 1985), may show differences that will make it possible to relate the repair to specific biochemical mechanisms.

REFERENCES

Braby, L. A., and J. M. Nelson. 1985. "Linear Energy Transfer (LET) Effects on Repair Rate." In *Physical Sciences*. Part 4 of

FIGURE 2. Split-Dose Survival with Proton Irradiation at Two Dose Levels

Pacific Northwest Laboratory Annual Report for 1984 to the DOE Office of Energy Research. PNL-5500 PT4, Pacific Northwest Laboratory, Richland, Washington, pp. 33-34.

Cox, J. L., and G. D. Small. 1985. "Isolation of a Photoreactivation-Deficient Mutant of *Chlamydomonas*." *Mutation Res.* 146:249-255.

Palmer, J. D. 1985. "Comparative Organization of Chloroplast Genomes." *Ann. Rev. Genetics* 19:325-354.

• 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 minimize biological variability. Cells not progressing through the cell cycle are needed in order to study the effects of dose protraction; cells in specific parts of the cell cycle are required in order to determine the changes in biochemical mechanisms that 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.

RE-ENTRY OF STARVED PLATEAU-PHASE CHINESE HAMSTER OVARY CELLS INTO THE REPLICATIVE CYCLE

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

Kinetic differences between fed and starved Chinese hamster ovary (CHO) cells have been demonstrated. We have further characterized these differences by subculturing 14-day-old plateau-phase cells and following them as they progressed through their first post-plateau-phase cycles. Deoxyribonucleic acid (DNA) distributions were determined by flow-cytometric (FCM) analysis of fixed trypsinized cells, stained by the acriflavine-Feulgen procedure. About 83% of the starved populations are found in G(1) phase. These cells re-enter the replicative cycle almost immediately, passing into S phase within 11 hours, and into G(2) phase within 18 hours. The remaining 17% initially comprise about 2/3 (~10%) S-phase and 1/3 (~7%) G(2)-phase cells. Both of these subpopulations progress rapidly through mitosis, apparently producing large numbers of DNA-deficient daughter cells as they do so. In contrast, fed cultures consist of a mixture of cycling and noncycling cells. The noncycling cells appear very similar to those observed in starved cultures. The cycling populations, however, progress peristaltically in an exceptionally synchronous manner, reflecting the intermittent feeding regimen. These nonexpanding, yet actively growing fed populations continue to produce nonviable daughters, which are later observed as Feulgen-positive debris.

Manuscripts reporting these FCM findings have been submitted for review. These papers demonstrate the accumulation of nonprogressing cells in both G(1) and G(2) phases, and confirm the existence of nonsynthesizing cells within S phase (i.e., nonprogressing cells that have S-phase DNA content). Also reported is the finding that fed plateau-phase cultures contain a significant growing population, which contributes large amounts of karyorrhectic debris to the system, apparently as a consequence of continuous production of DNA-deficient cells.

REMOVAL OF RADIATION DAMAGE BY SUBPOPULATIONS OF PLATEAU-PHASE CHINESE HAMSTER OVARY CELLS

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

Centrifugal elutriation techniques have been applied to many cell systems for separation of asynchronous or mixed populations, according to cellular cross-sectional area. Cells of the same cell-cycle age tend to have similar physical dimensions and densities. Although the correlation between DNA content and cell size is far from perfect, these methods are still suitable for producing populations significantly enriched in cells of a given cell-cycle age.

Our stationary-phase Chinese hamster ovary (CHO) cells are composed primarily of G(1)-phase cells (83%), with the remainder comprising both G(2) and S phases. A deoxyribonucleic acid (DNA) distribution of this starting population is shown in Figure 1a.

FIGURE 1. Typical DNA Distributions for Unfractionated, Starved Plateau-Phase Cells (a) and for Two Fractions of the Same Sample (b), Separated by Centrifugal Elutriation. The distribution in the upper panel shows about 83% of the population in G(1) phase, 10% in S phase, and 7% in G(2) phase. In the lower panel, the first fraction (solid line) contains smaller cells and contains about 99% G(1)-phase cells. A significant increase in the concentration of larger cells is apparent in a later fraction (dashed line), where the G(2)- and S-phase component represents about 50% of the population. Cells had been fixed with 70% ethanol and stained with propidium iodide.

Starting with such cultures, we are able to produce fractions enriched in G(1) and non-G(1)-phase (i.e., G(2)+S) cells. With care we have even been able to separate G(2)-phase from S-phase cells.

A relatively pure (99%) population of G(1)-phase cells can be obtained in this manner (Figure 1b, solid line). The G(2)- and S-phase cells can also be concentrated, but to a lesser extent. Although specific cases are frequently better, G(2) + S-phase non-G(1)-phase cells are generally enriched from about 17% to around 50% (Figure 1b, dashed line). Removal of radiation damage by cells in each of these fractions was studied in

split-dose experiments. Results of a typical experiment of this sort are shown in Figure 2. To date, we have observed no significant differences in cellular repair rate, either among the individual fractions or between the fractionated and unfractionated data sets. This suggests, therefore, that each of the repair processes found in stationary-phase cells is cell-age independent. That is, both processes probably operate concurrently in each cell regardless of the cell's position in the interphase cell cycle.

However, as has long been known, cellular radiation sensitivity does change rapidly and considerably as the cells progress from one phase to the next through the cell cycle. Since the rate of damage removal appears invariant, the change in survival must reflect the efficiency of producing that damage. We should also be able to draw some conclusions regarding damage production from these data. The damage accumulation model (Roesch 1978) considers the production of both single- and two-event types of damage, with the exponential removal of the latter. Each damage process is treated independently; in this model A is a constant associated with the probability of producing two-event damage, while C is a similar constant associated

FIGURE 2. Split-Dose Survival of Unfractionated, Starved Plateau-Phase Cells and Two Fractions Derived from the Same Sample by Centrifugal Elutriation, Plotted Against Time Between Irradiations. There is no striking difference among the unfractionated sample (solid line), the G(1)-phase cell fraction (small dashes), and the enriched S- and G(2)-phase cell fraction (large dashes). Differences that are apparent, particularly in the early data, are probably not significant.

with production of single-event damage. Changes in the relative values of A or C would be observed as changes in the shape of the survival curve. As an example, assume, for simplicity, only a single repair process. If there were no change in the probability of producing two-event damage, A, i.e., the dose-squared component, but the probability of producing the single-event damage, C, varied through the cell cycle, we would expect a significant change in asymptote on a semilog survival curve.

As shown in Figure 3a, a 50% change in the value of C leads to a change in survival of

FIGURE 3. Hypothetical Split-Dose Survival Curves, Predicted by the Damage Accumulation Model (Roesch 1978), That Would Result If the Single- or Two-Event Factor Varied. To simplify the situation, only a single repair process has been assumed. (a) shows the effect of changing the probability of producing the single-event damage (C) while holding the probability of producing the two-event damage (A) constant. In (b), A has been allowed to vary. In this case, the value of C has also been varied in order to bring the final survival to the same level.

about an order of magnitude. Similarly, if C were to remain constant while A was varied, the relative amount of damage being removed would vary accordingly. As shown in Figure 3b, a pronounced change in the intercept would be observed after the survival curves are normalized to the same final amount of damage, i.e., to the same survival level. Such effects were not observed in these experiments. However, it is possible that a relatively small change in C, coupled with a somewhat larger change in A, might be hidden within the resolution of the data. Similar changes would be reflected in the survival curve when two repair processes are considered. If the rate of both of the processes and the amount of damage available for repair by one of them were held constant while the amount repaired by the other varied, the form of the survival curve would change (see Figure 4). The experimental data

FIGURE 4. Hypothetical Split-Dose Survival Curves for a System Having Two Repair Times That Would Result If Only the Two-Event Factor Were Varied. In (a) only the probability of producing damage that would be removed by the faster repair process is allowed to change. In (b), only the probability associated with the slower process is allowed to change.

are inconclusive; however, they do suggest that production of one or another sort of damage probably dominates during specific phases of the cell cycle, while the capacity for removal of all types of damage remains relatively constant. This work will continue during the next year.

REFERENCE

W. C. Roesch. 1978. "Models of Radiation Sensitivity in Mammalian Cells." In Third Symposium on Neutron Dosimetry in Biology and Medicine. Ed. G. Berger and H. G. Ebert. Commission of the European Communities, Luxembourg, pp. 1-27.

KINETICS OF C3H 10T1/2 MOUSE CELLS

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

The 10T1/2 cell line has been brought to PNL because of its usefulness as an in vitro biological system for transformation assay, and because a fast repair process in response to irradiation has been observed in these cells (Malcolm and Little 1979). The cell line was originally established from embryonic C3H mouse tissue and characterized by Reznikoff, Brankow, and Heidelberger (1973). Its full name is C3H/10T1/2 Clone 8, American Type Culture Collection (ATCC) number CCL226. Before using the cells in radiobiological studies, it was important to observe the growth kinetics of our cultures and verify their similarity to those reported by other laboratories in which they are used, so that our subsequent measurements could be compared with and added to the body of scientific work already compiled.

Several frozen vials of 10T1/2 cells were received in January 1986 from the ATCC and were immediately stored in liquid nitrogen. One of the vials was thawed and the contents were used to initiate monolayer culture. The

optimal freezing protocol* was determined for our cultures, and then suitable numbers of passage 10 cells were grown up and frozen for eventual experimental use.

To become familiar with the cell morphology and cloning characteristics, we conducted an experiment in which a number of flasks were seeded with about 350 cells each. Each day, starting with the second day after plating, a flask was stained and the number of clones plus the number of cells in each clone were counted. There are several types of cell morphologies within the population, some initial cause for concern. However, the plating efficiency was determined to be an average of $28.6 \pm 2\%$ for days 5 to 8 after plating (shown in Figure 1), which is very close to Reznikoff's value of 27.8% and within the range of 25% to 30% reported in the literature today. The generation time at the period of fastest replication was found to be 15.5 hours, also virtually the same as that reported by Reznikoff.

Flow cytometry (FCM) studies were begun to assess cell cycle characteristics. Again, a number of flasks were seeded, but this time with a large number of exponentially growing cells. Daily, a flask of cells was fixed and stained from FCM. Figure 2 shows deoxyribonucleic acid (DNA) histograms of days 1, 3, 4, and 11. Cell cycle fractions were analyzed using a program developed by Dean (1985). Notice that on day 1, 85% of the cells are in G1 phase. This may indicate that the plating efficiency of G1 cells is greater than that of the other fractions. Unfortunately, the original seeding culture was not also sampled for DNA content, so there is no baseline for comparison. Day 3 showed the largest percentage of cells in G2M phase (22%); day 4 showed the highest fraction of S phase (52%). By day 11, cell cycle activity had slowed to the point at which 88%

*Ten percent dimethyl sulfoxide (DMSO) was added to the culture medium; prepared vials were frozen for 2 hours in a -76°C freezer and then transferred to a liquid nitrogen dewar.

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FIGURE 1. Plating Efficiency Was Determined to Be an Average of 28.6% for Days 5 to 8 After Seeding. A "clone" in this experiment was defined as any number of healthy cells growing as a group, including groups of one, especially in the first days after seeding.

of the cells were found to be in G1. These measurements indicate that the cultures are probably more or less synchronized at their initiation by plating efficiency effects, that they maintain a loose synchrony by phases through about the eighth day (data not shown), and that before at least the eleventh day they have begun to pile up in G1 phase.

REFERENCES

Dean, P. N. 1985. "Methods of Data Analysis in Flow Cytometry." Chapter 6 in Flow Cytometry: Instrumentation and Data Analysis. Ed. M. VanDilla, P. Dean, O. Laerum, and M. Melamed. Academic Press, Orlando, Florida, pp. 195-223.

FIGURE 2. Numbers of Cells in the Population with Specific Amounts of DNA, as Measured by Flow-Cytometric Analysis

Malcolm, A. W., and J. B. Little. 1979. "Rapid Recovery in Plateau-Phase Mammalian Cells." Radiat. Res. 80:38-48.

Reznikoff, C. A., D. W. Brankow, and C. Heidelberger. 1973. "Establishment and Characterization of a Cloned Line of C3H Mouse Embryo Cells Sensitive to Post Confluence Inhibition of Division." Cancer Res. 33:3231-3238.

• Modeling Cellular Response to Genetic Damage

This project is directed toward achieving a better understanding of the mechanisms by which damage induced in mammalian cells by physical and chemical carcinogens produces lethal and mutagenic effects. Our results continue to show the integrated nature of cellular response to radiation or chemical insult. Expression of deoxyribonucleic acid (DNA) damage as either loss of reproductive capacity or mutation is modulated by intracellular pools of precursors used in DNA synthesis. This is probably due to the role of these precursors in regulating the initiation and/or rate of DNA replication which modifies the extent to which DNA damage is repaired. In some cases, carcinogens appear to induce pool imbalance as well as DNA damage. The goal of this project is to elucidate and model the impact of perturbations in cellular metabolism on the cellular response to DNA damage.

MODULATION OF RADIATION-INDUCED MUTATION FREQUENCIES AND DNA SYNTHESIS BY DEOXYCYTIDINE IN CHINESE HAMSTER OVARY CELLS

C. N. Newman

Previously reported experiments (Newman 1985) established roles for DNA synthesis and the inhibition of DNA synthesis in the expression of radiation-induced mutations. This year, these experiments were continued and the study was expanded. Two different Chinese hamster ovary (CHO) cell lines were examined. Most experiments utilized CHO-K1 cells from this laboratory (designated CHO-K1PNL); however, supporting preliminary data from CHO-K1 cells obtained from the laboratory of Dr. P. A. Jeggo, Mill Hill Laboratory, London, U.K. (designated in this report as CHO-K1PJ) were also obtained. Experimental measurements were obtained for cells in one of two growth media: 1) medium A, which consisted of Ham's F-12 containing 10% fetal calf serum; or 2) medium B, which consisted of medium A containing 2 mM deoxycytidine (CdR). The presence of CdR differentially affected the nature of DNA replication, the inhibition of replication by radiation, and the corresponding mutation frequency responses. A unifying hypothesis interrelating these phenomena is offered.

Figure 1 illustrates the dose-response relationship of ultraviolet (UV) radiation fluence and mutation to 6-thioguanine resistance (TG^R) in CHO-K1PNL cells growing in each of the two media. In the presence of CdR (medium B), cells exhibit up to a 20-fold lower mutation frequency response than cells growing in the absence of CdR (medium A). A comparable suppression in the frequency of UV induction to ouabain resistance also occurs when these cells are in medium B (not shown).

FIGURE 1. Induction of 6-Thioguanine-Resistance (TG^R) by UV Radiation in CHO-K1PNL in Medium A (●) or Medium B (○).

This result suggests that the effect of CdR on mutation-induction is not locus-specific. Moreover, the phenomenal reduction in the mutation response in the presence of CdR is also seen following exposure to ionizing radiation (as illustrated in Figure 2), which implies that the effect of CdR is not mutagen-specific.

Table 1 compares the mutation response of CHO-K1PNL and CHO-K1PJ to a single UV fluence ($7.5 Jm^{-2}$). Mutation induction frequencies in medium A are much lower for CHO-K1PJ than for CHO-K1PNL and are, in fact, similar to those reported for other CHO-K1 cell lines (Newman and Miller 1985). However, in the presence of 2 mM CdR (medium B), and in contrast to the effect on CHO-K1PNL, an

FIGURE 2. Induction of 6-Thioguanine-Resistance (TG^R) in CHO-K1PNL by 5-Gy, 1.5-MeV Electrons in Medium A (●) or Medium B (○).

TABLE 1. UV-Induced Mutation-Induction

Cell	Medium	$TG^{R(a)}$ Mutations/ 10^5 Survivors
CHO-K1PNL	A	142
	B	7
CHO-K1PJ	A	20
	B	49

(a) Resistance to 10 μ M 6-thioguanine.

approximately 2.5-fold greater UV-induced mutation frequency is observed in these cells.

It is likely that a DNA precursor pool imbalance was caused by the presence of CdR (Bianchi and Celotti 1985). The possibility was therefore considered that this imbalance modulates mutagenesis by differentially perturbing the regulation of replicative DNA synthesis and/or repair responses in the two cell lines exposed to irradiation.

Cell radio-pulse incorporation and alkaline sucrose gradient velocity sedimentation studies were employed to ascertain whether exogenous CdR differentially alters DNA synthetic activity in control and/or in irradiated CHO-K1PNL and CHO-K1PJ cells. Results of CHO-K1PNL cell incorporation studies, reported earlier (Newman 1985), are shown in Figure 3. Within two hours after exposure to 10 Jm^{-2} UV (Figure 3a) or 15 Gy ionizing radiation (Figure 3b), the radio-pulse incorporation by irradiated cells in medium B is reduced to 22% or 51% of the unirradiated value for UV or ionizing radiation, respectively; in contrast, corresponding incorporation by irradiated cells in medium A is reduced to 60% or 84% of the unirradiated value for UV or ionizing radiation. Thus, radio-pulse incorporation by cells in medium B appears to be 2.7 or 1.6 times more sensitive to the inhibitory effects of UV or ionizing radiation. Furthermore, an approximate extrapolation of the lines generated by data points beginning two hours after exposure apparently indicates a correspondingly longer period of recovery to the control incorporation value by these cells in medium B.

The results of incorporation studies in UV-irradiated CHO-K1PJ are displayed in Figure 4. In these cells at two hours after exposure, there is an approximately three-fold difference in [3H]-thymidine incorporation in the two media; however, the greater inhibition and longer recovery period is now observed in cells grown in medium A (17% of control) rather than medium B (39% of control) as in the other cell line.

FIGURE 3. Inhibition of DNA Synthesis by UV Radiation in CHO-K1PNL in Medium A (●) or Medium B (○). Cells grown in the presence of $[^{14}\text{C}]$ -thymidine were exposed to 10 Jm^{-2} UV radiation, then periodically pulse-labeled with $[^3\text{H}]$ -thymidine. Dashed line represents 1.32-fold compensatory adjustment of unirradiated medium A control incorporation due to a 1.32-fold more rapid DNA chain polymerization rate in unirradiated cells in medium B (see Figure 6 and text).

Time-dependent changes in profiles of pulse-labeled DNA on alkaline sucrose gradients were employed to measure and compare nascent DNA chain growth processes in the two media (Kapp and Painter 1982). Ultracentrifugation

FIGURE 4. Inhibition of DNA Synthesis by UV Radiation in CHO-K1PNL in Medium A (●) or Medium B (○). Protocol is identical to that described in Figure 3.

of $[^3\text{H}]$ -thymidine pulse-labeled DNA as a function of post-label ("chase") time was used, adopting a standardized centrifugation protocol that was modified from an earlier report (Newman 1985) and now consistent with the technical recommendations for ascertaining DNA molecular weight, as described previously (Elkind 1971; Hutchinson 1981). Typical DNA profiles obtained in this manner for chase periods of 15 minutes and 45 minutes following a 5-minute $[^3\text{H}]$ -thymidine-labeling period of CHO-K1PNL cells in each growth medium are illustrated in Figure 5. In this, as in similar experiments with different chase periods (not shown), $[^3\text{H}]$ -DNA from cells grown in medium B sediments more rapidly than does that from cells grown in medium A. This phenomenon suggests that a more rapid DNA chain elongation and/or replicon maturation rate occurs in cells in medium B. In contrast, the sedimentation of

FIGURE 5. Alkaline Sucrose Gradient Profiles of [^3H]-Thymidine Pulse-Labeled DNA for CHO-K1PNL Grown in Medium A (closed symbols) or Medium B (open symbols). Cells were labeled for 5 minutes, then allowed to grow for 15 minutes (■, □) or 45 minutes (▲, △) before harvest and analysis. X represents continuously (72-hour) labeled cellular DNA. Cells were x-rayed (7.5 Gy) before analysis and centrifugation at 20°C, 6000xg for 21.25 hours.

pulse-labeled CHO-K1PJ DNA is always greater when cells are grown in medium A (not shown).

Relative number average molecular weights, Rel Mn, were calculated for each sedimentation profile and are each expressed as the ratio of the pulse-labeled DNA ($\text{Mn}[^3\text{H}]$) to the uniformly labeled reference DNA ($\text{Mn}[^{14}\text{C}]$) standard. In Figure 6 these Rel Mn values are each plotted as a function of the corresponding chase time for unirradiated and for UV-irradiated (10 Jm^{-2}) cells. The slopes of the straight lines obtained from unirradiated cells in the two media reflect the steady-state average DNA chain growth rates. For CHO-K1PNL cells in medium A and medium B, these values are 2.56×10^{-3} and 3.38×10^{-3} , respectively. When CHO-K1PJ cells are irradiated, however, similar slopes (2.04×10^{-3} for medium A and 2.09×10^{-3} for medium B) are obtained for up to 60 minutes following exposure of these cells to UV radiation.

The slope generated from Rel Mn data for unirradiated CHO-K1PJ cells in medium A is substantially (2.3-fold) greater than for the

FIGURE 6. Relative DNA Chain Polymerization Rates of CHO-K1PNL (●, ○, ▲, △) and CHO-K1PJ (■, □, ▼, ▽). Number average molecular weights were calculated for pulse-labeled (^3H) and continuously labeled (^{14}C) DNA obtained from sucrose gradient profiles (Figure 5). Relative molecular weights, Rel Mn, are expressed as $\text{Mn}[^3\text{H}]/\text{Mn}[^{14}\text{C}]$ and are plotted as a function of post-pulse-labeling growth period. The slopes of the straight line obtained from unirradiated cells reflects the steady-state average DNA chain growth rate. Closed symbols, unirradiated cells; open symbols, cells were exposed to 10 Jm^{-2} UV radiation. (▲, ▽, ▼, ▽) medium A; (●, ○, ■, □) medium B.

corresponding CHO-K1PNL culture. For unirradiated CHO-K1PJ cells in medium A and medium B, slopes are 5.9×10^{-3} and 3.2×10^{-3} , respectively; for UV-irradiated CHO-K1PJ cells in medium A or medium B, dissimilar values of 2.48×10^{-3} or 1.29×10^{-3} are also obtained. Note that the latter values each represent about 40% of the respective polymerization rate in unirradiated cells. Thus, there appears to be no medium-dependent effect of UV radiation on replicon chain growth in those cells. Therefore, something other than replication fork blockage by photoleisions must account for the medium-dependent 2.2-fold difference in relative [^3H]-thymidine incorporation by UV-irradiated CHO-K1PJ (1 hour postirradiation, Figure 4).

To ascertain whether UV radiation differentially affects the number of synthesizing replicons in each of the two media for CHO-K1PNL cells, earlier measurements of radio-pulse incorporation by UV-irradiated

cells in medium A (Figure 3) are re-evaluated, but now with consideration given to the findings of different chain growth rates in control cultures (Figure 6). A normalization factor of 1.32 is applied to the unirradiated control incorporation value, medium A, to compensate for that portion of incorporation occurring as a consequence of the apparent 1.32-fold faster DNA chain growth rate of unirradiated cells in medium B (Figure 6). The result of this adjustment on apparent incorporation by irradiated cells in medium A, shown as a broken line in Figure 3, is to reveal the effects of irradiation only on the relative number of active replicons in the two media. When the incorporation data are examined by this method, a two-fold greater inhibition in UV-induced incorporation is still observed in cells grown in medium B, in comparison to the response of cells in medium A. We interpret this to mean that, compared to medium A, about half as many replicons in medium B that would have initiated synthesis following irradiation actually do so. Since similar relative inhibition of DNA chain elongation occurs in CHO-K1PJ in both media (Figure 6), dissimilarities in the incorporation responses of UV-irradiated cells in the two media (Figure 4) might also be attributable to medium-dependent perturbations in postirradiation replicon initiations.

The foregoing results indicate that UV radiation appears to inhibit postirradiation replicon initiation events and results in reduced mutation frequencies when cells are grown in the medium that promotes the more rapid rate of polymerization. While we do not yet understand the mechanisms responsible for these effects of medium on replication, we propose that either 1) premutagenic lesions in uninitiated replicons are more

efficiently repaired than lesions in partially replicated DNA, where bulky replication fork complexes are blocked by the presence of photolesions, and/or 2) attempts to bypass unrepaired damage in partially replicated replicons are error-prone.

REFERENCES

- Bianchi, V., and L. Celotti. 1985. "Accuracy of UV-Induced DNA Repair in U-79 Cells with Imbalance of Deoxynucleoside Pools." Mutation Res. 146:277-284.
- Elkind, M. M. 1971. "Sedimentation of DNA Released from Chinese Hamster Cells." Biophys. J. 11:502-520.
- Hutchinson, F. 1981. "The Dependence of DNA Sedimentation on Centrifuge Speed." In Molecular Mechanisms for Repair of DNA, Part B. Ed. P. C. Hanawalt and R. B. Setlow. Plenum Publishing Corp., New York.
- Kapp, L. N., and R. B. Painter. 1982. "DNA Replication Fork Movement Rates in Mammalian Cells." Int. Rev. Cytol. 80:1-25.
- Newman, C. N., and J. H. Miller. 1985. "Mechanism of UV-Induced Deoxynucleoside Triphosphate Imbalance in CHO-K1 Cells." Mutation Res. 145:95-101.
- Newman, C. N. 1985. "Regulator of DNA Synthesis by Deoxynucleoside Pools and the Induced Mutation Response." In Physical Sciences. Part 4 of Pacific Northwest Laboratory Annual Report for 1985 to the DOE Office of Energy Research. PNL-5750 PT4, Pacific Northwest Laboratory, Richland, Washington, pp. 43-46.

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PUBLICATIONS

- DuBois, R. D. 1986. "A Coincidence Study of Doubly Differential Cross Sections: Projectile Ionization in He^+ - He Collisions." Phys. Rev. Lett. 57:1130-1132.
- DuBois, R. D. 1986. "Charge Transfer and Ionization Occurring in Proton- and Helium Ion-Atom Collisions." Prepared for Joint US-Mexico Workshop on 2e Phenomena.
- DuBois, R. D. 1986. "Ionization and Charge Transfer in He^{2+} - Rare-Gas Collisions." In Phys. Rev. A 33:1595.
- DuBois, R. D. 1986. "Recent Studies of Simultaneous Ionization and Charge Transfer in Helium Ion-Atom Collisions." In Nuclear Instruments and Methods, in press.
- DuBois, R. D., and S. T. Manson. 1986. "A Study of Multiple Ionization Channels in Proton-Atom Collisions." Submitted to Physical Review A.
- Metting, N. F., and L. A. Braby. 1986. "A Comparison of Microscopic Dose with Average Dose Near High Energy Heavy Ions." Nuclear Instruments and Methods, in press.
- Metting, N. F., and L. A. Braby. 1986. Measurement of Energy Distributions Near High Energy, Heavy Ion Tracks. PNL-5950 (UC-48).
- Miller, J. H., W. E. Wilson, S. T. Manson, and M. E. Rudd. 1986. "Differential Cross Sections for Ionization of Water Vapor by High-Velocity Bare Ions and Electrons." Accepted for publication in J. Chem. Phys.
- Nelson, J. M., L. A. Braby, M. K. Lien, and C. A. Ratcliffe. 1986. "Computer Recognition of Mammalian Cells." In Cell Tissue Kinetics (Abstract in press).
- Nelson, J. M., L. A. Braby, N. F. Metting, and W. C. Roesch. 1986. "Removal of Radiation Damage by Subpopulations of Plateau-Phase CHO Cells, Fractionated by Centrifugal Elutriation." In Cell Tissue Kinetics 19(2): pages not available (Abstract in press).
- Newman, C. N., and H. M. Hagler. 1986. "Degradation or Consumption of Exogenous Thymidine in Absence or Presence of Exogenous Deoxycytidine: Effect on DNA Chain Elongation and [^3H]-Thymidine Incorporation in Control and UV-Irradiated CHO-K1 Cells." Submitted to Biochemical Journal.
- Roesch, W. C. 1986. "Problems Related to the Critical Depth of Skin." In Rad. Prot. Dos. 14:91-93.
- Wilson, W. E., and T. L. Criswell. 1986. "Microscopic Track Structure of Equal-LET Heavy Ions." In Proceedings of COSPAR-86 Conference, Toulouse, France, June 30-July 7, 1986.

PRESENTATIONS

Braby, L. A., N. F. Metting, J. M. Nelson, and H. D. Thames. "Unrepaired Damage in Plateau Phase CHO Cells: Potentially Lethal or Sublethal." Presented at the 34th Annual Meeting of Radiation Research Society, Las Vegas, Nevada, April 13-17, 1986. (PNL-SA-13557 A)

DuBois, R. D. "Charge Transfer and Ionization Occurring in Proton- and Helium-Ion-Atom Collisions." Presented at Joint US-Mexico Workshop on 2e Phenomena, Cuernavaca Mexico, January 8-11, 1986. (PNL-SA-13602)

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DuBois, R. D. "Recent Studies of Simultaneous Ionization and Charge Transfer in Helium Ion-Atom Collisions." Invited paper presented at the Ninth Conference on the Application of Accelerators in Research and Industry of the American Physical Society, Denton, Texas, November 10-12, 1986. (PNL-SA-14084 A)

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