COO-2486-366

INVESTIGATIONS OF THE BIOLOGICAL EFFECTS OF RADIATION: A MULTI-DISCIPLINE APPROACH

Progress Report
for Period Sept. 1, 1976 -Aug. 31, 1977

Hymer L. Friedell, M.D., Ph.D.

Department of Radiology
Case Western Reserve University
Cleveland, Ohio

September 1, 1977

Prepared for

THE U.S. ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION
UNDER CONTRACT NO. EY-76-S-02-2486.A001

DISCLAIMER

NOTICE

PROGRESS REPORT

For Period Sept. 1, 1976 - Aug. 31, 1977

Table of Contents

Professional Staff 1976-1977

(Percentage of effort devoted to Contract in parentheses)

Hymer L. Friedell, M.D., Ph.D., Director, Department of Radiology (10%)

Oddvar F. Nygaard, Ph.D., Director, Division of Radiation Biology (20%)

Project No. 1: Earle C. Gregg, Ph.D., Principal Investigator (20%)
George Bakale, Ph.D. (80%)
William H. Voelker, Ph.D. (20%)

Project No. 2: Eugene N. Brewer, Ph.D., Principal Investigator (80%)
(20% of time devoted to project No. 4)

Project No. 3: Helen H. Evans, Ph.D., Principal Investigator (40%)
Thomas E. Evans, Ph.D. (40%) (Terminal year, Final Report to be submitted later).

Project No. 4: Ronald C. Rustad, Ph.D., Principal Investigator (40%)
Nancy L. Oleinick, Ph.D. (20%) (Terminal year, Final Report to be submitted later).

PUBLICATIONS AND REPORTS

A. Prepared during earlier budget periods, published during the period May 1, 1976 - August 31, 1977

COO-2486-354 Radiation-induced Mitotic Delay in Eggs of Sea Urchins and Marine Annelid Worms (Abstract)
Ronald C. Rustad and Gomathy Viswanathan
Radiat. Res. 67, 630 (1976).

COO-2486-355 Radiation-induced Mitotic Delay in Eggs of a Marine Annelid Worm (Abstract)
Gomathy Viswanathan and Ronald C. Rustad
J. Cell Biol. 70, 2, 177a (1976).

COO-2486-356 Deoxynucleoside Metabolism in Physarum polycephalum: The Effect of Cycloheximide and of Ionizing Radiation (Abstract)
Helen H. Evans, Sandra R. Littman and Thomas E. Evans
Fifth Myxomycete Conference, Gainesville, Florida, November, 1975.

COO-2486-357 Studies on Radio-tracer Incorporation Into Amoebae of P. polycephalum (Abstract)
Thomas E. Evans, Lorna C. Leicht and Helen H. Evans
Fifth Myxomycete Conference, Gainesville, Florida, November, 1975

COO-2486-358 Nucleotide Metabolism in Physarum polycephalum: The Effect of Ionizing Radiation (Abstract)
Helen H. Evans, Sandra R. Littman and Thomas E. Evans
Radiat. Res. 67, 531 (1976).

COO-2486-361 Negative Ion-Molecule Reactions in Liquid Argon Following Electron Capture by N_2O. (Abstract).
George Bakale, Ulrich Sowada and Werner F. Schmidt
Presented at Electrons in Fluids Conference, Banff, Alberta, Canada, September 5, 1976.

COO-2486-362 Structural Effects on the Electron Attachment Rates of Nitro-aromatic Molecules and Implications to Radiation Sensitization (Abstract)
George Bakale, Earle C. Gregg and Richard D. McCreary
Radiat. Res. 67, 607 (1976).

COO-2486-363 Influence of Non-Electronegative Molecules on the Low-Field -Mobility of Excess Electrons in Liquid Rare Gases (Abstract)
Ulrich Sowada, Werner F. Schmidt and George Bakale
Presented at Electrons in Fluids Conference, Banff, Alberta, Canada, September 5, 1976.

COO-2486-364 Differential Modification of Synthesis Rates for Ribosomal and Messenger RNAs in Gamma-Irradiated Tetrahymena pyriformis (Abstract)
Susan G. Ernst, Nancy L. Oleinick and Ronald C. Rustad
Radiat. Res. 67, 530 (1976).

B. Prepared during the period May, 1976 - August 31, 1977

Negative Ion-Molecule Reactions in Liquid Argon Following Electron Capture by N_2O
George Bakale, Ulrich Sowada and Werner F. Schmidt
Can. J. Chem. 55, 2220-2223 (1977).

The Influence of Non-Electronegative Molecules on the Mobility of Excess Electrons in Liquefied Rare Gases and Tetramethyl Silane
Ulrich Sowada, Werner F. Schmidt and George Bakale
Can. J. Chem. 55, 1885-1889 (1977).

Caffeine-Radiation Interactions and Mitotic Delay (Abstract)
Nancy L. Oleinick, Eugene N. Brewer and Ronald C. Rustad
American Society for Cell Biology Meeting, San Diego, California
November, 1977

Dependence of Electron Mobility on Electric Field Strength in Nonpolar Liquids
Ulrich Sowada, Werner F. Schmidt and George Bakale
High Energy Chemistry 10, 290 (1977) translated from Khimiya Vysokikh Energii 10, 323 (1976).

Electron Attachment to Nitro Compounds in Liquid Cyclohexane
George Bakale, Earle C. Gregg and Richard D. McCreary
Submitted to J. Chem. Phys.

Excess Electron Mobility in Liquid Dielectrics Near the Critical Temperature (Abstract)
W. Doldissen, G. Bakale and W.F. Schmidt
6th Int. Conf. on Conduction and Breakdown of Dielectric Liquids, Rouen, July, 1978

The Reduction of Radiation-induced Mitotic Delay by Caffeine: A Test of the Cyclic AMP Hypothesis
Nancy L. Oleinick, Eugene N. Brewer and Ronald C. Rustad
Submitted to Nature

A Simplified Growth Medium for Physarum polycephalum (Abstract)
E.N. Brewer and A. Prior
Physarum Newsletter, 8, 45 (1976).

IONIZATION IN LIQUIDS
(Project No. 1)

PROGRESS REPORT
covering the period of September 1, 1976 - August 31, 1977

Principal Investigator: E.C. Gregg, Ph.D.

Department of Radiology
Case Western Reserve University
Cleveland, Ohio

September 1, 1977

Prepared For

THE U.S. ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION
UNDER CONTRACT NO. EY-76-S-02-2486.A001

PERSONNEL

Principal Investigator:	E.C. Gregg, Ph.D., Professor 20% effort
Co-Principal Investigator:	G. Bakale, Ph.D., Assistant Professor 80% effort
Equipment Development:	W.H. Voelker, Ph.D., Assistant Professor 20% effort
Accelerator Operator:	R.D. McCreary 100% effort
Research Assistant:	Anthony Ho 100% effort

TABLE OF CONTENTS

Summary Progress Report

The quasi-free electron attachment rate, k_e, and mobility, μ_e, were studied in non-polar solutions using pulsed conductivity techniques. Measurements of k_e of >50 nitro compounds in liquids having μ_e ranging from <0.1 to 100 cm^2/volt sec at temperatures from -100 to +40°C indicated electron-dipole interactions are important in liquids having μ_e < 1 cm^2/volt sec. The Smoluchowski equation was modified to include electron-dipole interactions and calculated k_e's agreed with observations within ± 20 percent. The cellular enhancement ratio, CER, of nine of the nitro compounds were measured and a correlation between k_e and CER was found which was used to refine a model of cellular radiosensitization involving quasi-free electrons. Diffusion-controlled k_e's were observed for several carcinogens and in reversed micellar solutions. Field-dependent k_e's were measured in liquids having μ_e ranging from 10^{-4} to 500 cm^2/volt sec and were found to increase at $\mu_e \leq 1$ and decrease at $\mu_e > 70$ cm^2/volt sec with increasing field. The μ_e of liquid C_2H_6 was measured from -40°C through the critical temperature at fields up to 180 kV/cm and a transition from polaron to delocalized electron conduction was observed. A pico-second (ps) pulse conductivity technique was developed and hot electron and/or autoionization processes were observed in tetramethylsilane, TMS, 200 ps after the ionizing pulse. A dose, field, and polarity dependent conductivity spike having a lifetime of 100 ps was observed in TMS and is interpreted as a pre-breakdown phenomenon.

A. ELECTRON ATTACHMENT TO POLAR SOLUTES

I) Electron-Dipole Interaction

In the course of determining what factors influence the electron attachment rate to radiosensitizers, we discovered that the attachment rate depended strongly on the dipole moment of the electron acceptor. To clarify this effect, which has not been observed for solvated electron reactions, we measured the electron attachment rates of a series of 39 nitrocompounds that had dipole moments ranging from 0.5 to $\sim$ 7 Debye. A full description of this work is presented in the appended manuscript, "Electron Attachment to Nitro Compounds in Liquid Cyclohexane", and is summarized along with more recent studies of this subject in the following.

The rate constants were measured with an accuracy of $\pm$ 10 percent using the pulsed conductivity technique that we developed (1) and were found to range from 3 - 7 x $10^{12} M^{-1} s^{-1}$ in liquid cyclohexane at 20°C. These values are greater than the diffusion-controlled rate constant, k_d, of a non-polar solute in the same liquid (2,3), which is given by (4)

$$k_d = 4\pi (D_e + D_S)R \qquad [I]$$

where D_e and D_S are the diffusion coefficients of the electron

and solute, respectively, and R is the effective encounter radius, i.e. the electron-solute separation distance at which attachment occurs. In liquid cyclohexane at 20°C, D_e = 5.5 x $10^{-3}cm^2/sec$ (from the Nernst-Einstein equation for μ_e = 0.22 cm^2/volt-sec) and $D_e >> D_S$. For a non-polar solute of radius 3.5 Å, Eq. I yields $k_d = 1.5 \times 10^{12} M^{-1}s^{-1}$.

Modification of Eq. I to include both electron-induced dipole and electron-dipole interaction for electron attachment to polar solutes yields a larger value of R, namely:

$$R = \left[1/2\sqrt{\frac{\pi\epsilon kT}{e\,\mu_{eff}}}\right) \cdot \operatorname{erf}\left(\sqrt{\frac{e\,\mu_{eff}}{\epsilon kT}}\, r^*\right)\Big]^{-1} \qquad [II]$$

(see attached manuscript for details of derivation and nomenclature). The effective dipole moment, μ_{eff}, is dependent upon the electron-dipole orientation angle θ and is given by

$$\mu_{eff} = \mu \cos \theta \qquad [III]$$

where μ is the solute dipole moment.

Values of k_d and μ_{eff} were calculated for solute dipole moments ranging from 0.5 to 7 Debye and agreed with the measured rate constants for 36 to 39 monosubstituted nitrobenzenes and for several poly-substituted nitrobenzenes. From studies of the latter we conclude that steric effects did not inhibit the rate of electron attachment and that resonance-decoupled nitro-groups on the same solute serve as independent electron acceptor sites. This suggests that poly-nitro or nitrilo substituted aromatic compounds are good radiosensitizer candidates.

Another prediction made from this study of electron attachment to polar solutes is that the electron-dipole interaction should influence the attachment rate more strongly in liquids having a low electron mobility (e.g. cyclohexane, μ_e = 0.22 cm^2/volt-sec) than in a high mobility liquid such as tetramethylsilane (TMS, μ_e = 95 cm^2/volt-sec). This is based upon the dipole having more time to rotate into a favorable orientation in a low mobility liquid during the time that the electron is temporarily trapped by the solvent, whereas in TMS the electron remains free and moves through the liquid with a uniform thermal velocity of $\sim 2 \times 10^6$ cm/sec. With this average velocity the electron is within the dipole attraction "range" of 18 Å for an acceptor having a dipole moment of 7 Debye (see Table V of manuscript) for $\sim 10^{-13}$sec, which is less than the rotational period of molecules.

In order to test this hypothesis the electron attachment rates to ortho and para-dinitrobenzene (o- and p-DNB, dipole moments are 6.1 and 0.5 Debye respectively) in TMS were measured. In agreement with our prediction, the electron attachment rates to these two dinitrobenzenes in TMS are both $5 \pm 1 \times 10^{14} M^{-1} sec^{-1}$, whereas we observed a threefold difference in the attachment rates in cyclohexane.

These electron attachment rates in TMS are the highest attachment rates observed in a liquid at room temperature which suggests that these reactions are truly diffusion-controlled. Quantitative evidence of this may be obtained by substituting the measured attachment rate constant of $5 \times 10^{14} M^{-1} s^{-1}$ or

8.3×10^{-7} cm^3/sec and the diffusion coefficient of 2.4 cm^2/sec (from μ_e and the Nernst Einstein equation) into Equation I and solving for R. The effective encounter radius obtained is 2.8 Å which is in reasonable agreement with the hard core radius of 3.0 Å of toluene determined from viscosity measurements. Thus, electron-dipole interaction does not enhance the attachment rate in liquids in which the electron is not trapped for some fraction of its lifetime by the liquid.

Cyclohexane and TMS represent limiting cases of the effect of liquid structure on electron-dipole interaction. With these limits established, we are now studying electron attachment to polar solutes in liquids having intermediate electron mobilities. These include the solvents (μ_e, cm^2/volt-sec) cyclopentane (1.1), isoctane (5.5), neohexane ($\sim$10) and isoctane-TMS mixture (μ_e ranges from 5.5 to 95 cm^2/volt.sec, see Part 4 of this Section).

Another means of studying electron interactions with solutes and solvents is through the effect of temperature on the electron attachment rate. The activation energies of electron attachment to several substituted nitrobenzenes have been measured and are listed in Table 1. The activation energies are $\sim$3 kcal/mole for all of the nitro compounds studied which results from the activation energy of the mobility being 3 kcal/mole (5), i.e. from Equation I, k_d increases with temperature only through the increase in D_e (or μ_e) with temperature. The contribution to k_d due to the temperature dependence of R in Equation II is only 12 per cent in the 5 - 40°C temperature range that we studied for this solvent.

Table 1. Activation energy, E_{act}, of electron attachment to solutes in cyclohexane from 5-40°C.

Solute	E_{act}, kcal/mole
Nitrocyclohexane	2.8
Nitrobenzene	3.4
o-Dinitrobenzene	2.9
o-Nitrobenzonitrile	3.2
p-Nitrobenzonitrile	3.5
p-Fluoronitrobenzene	3.0
Carbon tetrachloride	2.8

The temperature dependence of k_D in TMS is different, however, for in this liquid, D_e is unchanged from +20 to -98°C. Thus, any change in k_d with T must be due to the R term in Equation II. The predominant temperature dependence in Eq. II is the T^{-1} pre-error function term which predicts an enhancement of the attachment rate by 50 per cent as the temperature decreases from +20 to -78°C. This is the dependence we observed for electron attachment to the nitro compounds listed in Table 2 with enhancement of the electron attachment rate at the lower temperature ranging from 10 to 90 per cent.

This result, however, is contradictory to our conclusion that electron-dipole interaction is negligible in TMS and, consequently, Eq. II should not apply to the electron attachment process. We are attempting to resolve this apparent paradox through studies of electron attachment to polar solutes in TMS and in isoctane-TMS mixtures at intermediate temperatures.

II) "Quasi-Free" Contrasted With "Dry" Electrons

Another study that we are presently conducting is to establish the correlation between the quasi-free electron and dry electron attachment processes. We had proposed that such a correlation between the two types of electrons should exist before any suggestion was made by others that quasi-free electron reactions in non-polar liquids may simulate dry electron reactions in aqueous solutions. In order to determine if the two-electronic species have similar reactive properties, we are studying the electron attachment rates of solutes in non-polar liquids that have already been studied in concentrated

Table 2. Electron Attachment Rate Constants of Nitro Compounds in Tetramethylsilane at 20 and -78°C.

Solute	$k \times 10^{-14}$ M^{-1} s^{-1} 20°C	-78°C
o-Dinitrobenzene	4.6	6.4
p-Dinitrobenzene	5.5	6.1
o-Nitrotoluene	0.82	1.2
p-Nitrotoluene	0.58	1.1
p-Nitroacetophenone	1.9	2.5

polar media (6,7).

Preliminary results from this study are presented in Table 3 which illustrates that solutes which attach dry electrons at high rates also attach quasi-free electrons very efficiently. Extension of this study to other solutes for which the dry electron attachment rates are known and to other non-polar liquids should result in an estimate of the mobility and diffusion coefficient of the dry electron. Knowledge of these transport parameters within an order of magnitude would be invaluable in improving the theory of the reactions that occur following interaction of ionizing radiation in biological systems.

B. ELECTRON ATTACHMENT AND BIOLOGICAL PROCESSES

I) Radiation Sensitizers

A more direct study of the role of electron attachment in hypoxic cellular radiosensitization was made in collaboration with Dr. C.L. Greenstock of Atomic Energy of Canada, Whiteshell Nuclear Laboratory. Dr. Greenstock measured the cellular enhancement ratios of three series of ortho-, meta- and para-nitro compounds for which we had measured the electron attachment rates. The results are summarized in Table 2 where one sees that the dinitro-compounds are the most effective hypoxic cell sensitizers with the ortho-compound being the most effective. This is in accord with our predictions as is the poor sensitization by the bromonitrobenzenes. The latter compounds were predicted to be poor sensitizers due to their forming relatively short-lived anions when attachment occurs (see Table 4). The enhancement ratio appears to involve both

Table 3. Comparison of Rates of Attachment of Quasi-free Electrons in Cyclohexane and of Dry Electrons in Ethanol to Various Solutes.

Solute	$k \times 10^{-10} M^{-1} s^{-1}$ Quasi-free	Dry[a]
Carbon Tetrachloride	300	1.2
Chloroform	300	1.1
Nitrobenzene	510	1.2
Acetophenone	360	1.2
Methyl Ethyl Ketone	0.7	0.6
Acetone	0.5	0.5

Table 4. Cellular enhancement ratio, C.E.R., electron attachment rate constant (in cyclohexane at 20°C), k, product anion lifetime, T, and logarithm of the product kT of substituted nitrobenzene compounds.

Sensitizer	C.E.R[a)]	$kx10^{-12}M^{-1}s^{-1}$	T, μsec[b)]	log kT, M^{-1}
o-dinitrobenzene	1.7	6.7	463	9.5
m-dinitrobenzene	1.6	5.0	537	9.4
p-dinitrobenzene	1.6	2.8	421	9.1
o-nitrobenzaldehyde	1.5	5.5	395	9.3
m-nitrobenzaldehyde	1.2	4.8	205	9.0
p-nitrobenzaldehyde	1.4	3.7	47	8.2
o-bromonitrobenzene	1.4	6.1	18	8.0
m-bromonitrobenzene	1.2	5.3	21	8.1
p-bromonitrobenzene	1.1	5.0	10	7.7

(a) Cellular enhancement ratios measured by C.L. Greenstock, private communication.

(b) Reference 19, Table 1

the electron attachment rate and the product anion lifetime and as a first attempt to determine the correlation function, the enhancement ratio was plotted as a function of the log of the product kT (Fig. 1). There appears to be a good correlation between these parameters for 7 of the 9 nitro compounds studied.

The two nitro compounds which deviate most from the correlation are o-bromonitrobenzene and m-nitrobenzaldehyde. Since cellular respiration is intimately related to the radiosensitization process due to oxygen depletion effects, we predict that the anomolous behavior of the two nitro-compounds is due to o-bromonitrobenzene inhibiting and m-nitrobenzaldehyde stimulating cellular respiration more efficiently than the other nitro compounds. An additional factor to consider is the cellular toxicity, and both cellular respiration and toxicity studies of the nine nitro compounds have been initiated in collaboration with Dr. J. Biaglow's group in our laboratory.

II) Carcinogenesis

Another biological process which appears to involve quasi-free electrons is carcinogenesis. Measurements of electron attachment rates to several carcinogens in non-polar liquids are summarized in Table 5 which illustrates that all of the carcinogens studied attach electrons at diffusion-controlled rates. This is in contrast to reaction rates orders of magnitude less for electron attachment to naturally occuring cellular components (8) and to several compounds recognized to be non-carcinogens such as caffein, nicotine, and ethanol (9).

The high attachment rate constants of carcinogens suggest

Table 5. Electron attachment rate constants of carcinogens in non-polar liquids at 20°C.

Carcinogen	$k \times 10^{-12} M^{-1} s^{-1}$ in solvent: n-hexane	c-hexane	i-octane
benzo-α-pyrene	1.3	4.0	12
2-nitrofluorene	1.3	4.8	36
4-nitroquinoline-N-oxide	0.9	2.1	10
chloroform	1.2	3.0	13
carbon tetrachloride	1.2	3.0	--

that this property could be used as a pre-screening test of suspected carcinogens. The main advantages of this pre-screening method compared to other screening tests are sensitivity and speed. The high electron mobility and attachment rates combine to make picogram quantities of a carcinogen detectable in analysis times of less than 20 minutes. A positive response from the pre-screening electron attachment test, which we've already found for many radiation sensitizers, would indicate a need for further study in one of the more time consuming and more expensive conventional screening tests that are available (10).

We hope to more firmly establish the electron attachment carcinogenicity correlation through measurements of the electron attachment rate constants of compounds clearly recognized as being either carcinogens or non-carcinogens. Among the non-carcinogenic drugs tested, nicotine was found to have an unusual electron attachment behavior, viz, both electron attachment to nicotine and electron detachment from the product anion occurred at about the same rate which resulted in an electron attachment-detachment equilibrium. Equilibria of this type generally are very temperature dependent which permits evaluation of the thermodynamic properties of the electron acceptor-donor (11,12).

We found a similar electron attachment-detachment equilibrium to occur with para-difluorobenzene (p-DFB) in normal and cyclo-hexane from 5 to 35°C. Computer analysis of the temperature

dependence of the equilibria by Dr. R.A. Holroyd of the Brookhaven National Laboratory Chemistry Department yielded an electron affinity for p-DFB of -0.34eV. This value is in marked contrast to the gas phase value of +0.18 eV which illustrates the marked influence of electron-solvent and anion-solvent interactions on the reaction properties of electrons.

The electron affinities of the ortho- and meta-difluorobenzenes are now being measured to determine the role of solute dipole moment on the electron affinity. Drugs having pharmacological properties similar to nicotine will be studied to determine if the electron attachment-detachment equilibrium is biologically important.

C. ELECTRON TRANSPORT IN BIOLOGICAL SYSTEMS

Although a better understanding of the electron attachment process is valuable in clarifying the roles that electrons play in biological processes, a more detailed knowledge of electron transport in biological systems is of equal importance. Cellular electron transport can be conveniently divided into three categories:

(i) intra-molecular transport within biomolecules such as proteins, RNA and DNA which appear to have semi-conductor properties (13,14,15).

(ii) electron transport through the structured, quasi-lattice multilayers of water bound to highly polar biomolecules and into the disordered bulk water, and

(iii) electron transport through structured layers of amphipathic molecules that constitute memebrane walls.

I) Intra-molecular Transport

Concerning the first category, we have applied the pulse-conductivity technique to directly measuring microsecond decay processes of charge carriers in strands of RNA and DNA; however, the observed currents were non-ohmic and varied greatly with different samples. We have designed an improved sample holder which will permit us to maintain the biomolecules under vacuum during the measurement which should improve reproducibility. Sample preparation techniques, also have been improved and these modifications may permit us to make the first direct measurements of the mobilities of electrons and holes in DNA and RNA.

II) Inter-molecular Transport

The second and most complex type of cellular electron transport could only be treated theoretically until recently since technology prevented our making direct observations of the properties of electrons occurring when the environment of the electron changes from a highly structured, pseudo-non-polar medium to one in which electron-dipole interactions predominate and the electron becomes fully solvated. This is the type of environmental change that an electron encounters after being ejected from a polar biomolecule such as DNA that has absorbed sufficient energy for ionization to occur.

The best experimental simulation of the changes of the electronic properties that occur during this solvent transition

are in studies being conducted at the Reactor Institute, Delft, The Netherlands, where A. Hummel and J.M. Warman recently have applied a pulsed microwave conductivity technique to studies of frozen aqueous solutions (16). Observation of a highly reactive, very mobile charge-carrier in the frozen solutions containing various solutes, including DNA, indicate that this charge-carrier is an unsolvated electron. We have maintained close contact with this group for years and anticipate directly collaborating with them in a joint study of determining the attachment and transport properties of electrons in frozen polar media.

These experimental studies should rigorously test the model that we derived to describe the charge transport properties of cellular media following an ionizing event in the cell (17,18). In this model the time and position dependency of the mobility of the ejected electron is considered as it loses energy to the medium which has a time and position dependent dielectric "constant" ranging from 3.5 to 80. The probability of the electron encountering and attaching to an electron affinic solute before becoming solvated is also considered as well as what subsequent electron reaction pathways are modified if attachment occurs. An abstract of the paper to be presented is appended.

III) Membrane Transport

The third category of electron transport related to biological systems that we studied was electron migration through membranes. Cellular membrane wall structure is simulated to a high degree in

the walls of micelles formed by surfactants in polar solvents (20). In non-polar solvents, a great variety of amphipathic molecules coalesce to form inverted or reversed micelles in which the hydrophobic "tails" of the solute extend into the non-polar solvent and the hydrophilic "heads" aggregate at the interior. The polar environment of the reversed micelle solubilizes polar solutes present in the solution which aids in stabilizing the entire aggregate. Details of the structure and catalytic properties of these unique macromolecules have been presented in a recent review (21).

We have observed reversed micellar formation of licithin, cholesterol, and chlorophyll in normal cyclohexane and in isoctane (18). Our most recent studies were directed at determining the electron transport properties of these species which are of particular interest because electron reactions play a key role in photobiological processes (15,22-24). Further, water incorporated in the inner reversed micelle pool is known to have considerable structure as is reflected by the solubilized water in Aerosol OT, sodium bis (2-ethylhexyl) solfosuccinate, having a dielectric constant of 2-4 (25). Electron reactions in this structured water should be another means of simulating radiation biological electronic processes.

In order to clarify these aspects of the electron transport modifying properties of reversed micelles, we wanted a reverse micelle system for study which had well defined physical properties. We chose Aerosol OT (AOT) which is known to have the structured water pool referred to above and which has a

known micellar weight and geometry in liquid hydrocarbons determined from ultracentrifugation, light scattering, and viscometric studies by Peri (26).

We observed two components in the decay of the ion current in AOT-normal hexane solutions varying in composition from 0.32 to 6 weight percent with an applied electric field ranging from 15 to 150 kV/cm. The major linear component yielded an ion mobility of $2.2 \times 10^{-4} cm^2$/volt sec and the minor component could only be assigned a lower limit of $>4 \times 10^{-5} cm^2$/volt sec due to the non-linear nature of the decay. Converting these mobilities to diffusion coefficients using the Nernst-Einstein equation yields values of the diffusion coefficients of 5.5×10^{-6} and $>1 \times 10^{-7} cm^2$/sec for the major and minor components, respectively. The diffusion coefficient of the major micellar component is in fair agreement with Peri's value of $3 \times 10^{-6} cm^2$/sec for Aerosol OT in isoctane (26). The micellar weight of this aggregate is ~9,000 which corresponds to 25 AOT molecules/micelle. No higher molecular weight aggregates were detected by Peri that corresponded to the slower decaying current we observed, but this may have been due to the different sensitivities of the experimental techniques.

In aqueous micellar solutions, hydrated electrons attach to non-polar solutes in micelles at diffusion-controlled rates (27) and appear to tunnel through the electrical double layer at the micelle-water interface (28). The reaction rate constants observed in these studies are in good agreement

with the rate constant of $2.5 \times 10^{10} M^{-1} s^{-1}$ calculated from diffusion theory for a micelle having a radius of 45 Å and with the hydrated electron having a diffusion coefficient of $4.5 \times 10^{-5} cm^2/sec$.

We attempted to compare the quasi-free electron attachment rate in AOT-isoctane solutions with the above studies but found that the electron attachment reaction was too fast for us to observe ($t_{1/2}$ <20 nsec). This may be due to analogous attachment processes occurring in the two systems but with the reaction rate of the quasi-free electron being ∿3000 times greater than that of the hydrated electron due to the diffusion coefficient of the former being greater than that of the latter by this factor. Consequently, a sub-micromolar concentration of an efficient electron attaching impurity would have reduced the quasi-free electron lifetime to <20 nsec in the AOT-isooctane solution but could have been tolerated in the aqueous micellar system.

We have improved our purification techniques and are now preparing to repeat the AOT-isooctane experiments. When we are able to observe electrons with a half-life >200 nsec in this system, polar electron acceptor molecules will be added to the solution and their electron attachment rate constants measured. Polar electron acceptors that we have already studied in pure non-polar liquids will be used and the influence of the micelle on the electron attachment rate of the acceptor will be determined. When this is established, other reverse micelles will be substituted for AOT in order

to determine the effect of micellar structure on the electron attachment and transport processes. The first micelles we will study are lecithin and chlorophyll since these will provide information on electron transport through cellular membranes and chloroblasts. Subsequent studies will include electron attachment to nitromididazole radiosensitizers in lecithin or other reversed micelles that simulate cellular membranes.

D. ELECTRON TRANSPORT AND ATTACHMENT PROCESS IN MODEL LIQUIDS

The studies described in the preceeding portions of this Section are all related to the role of electrons in biological processes and utilize knowledge of quasi-free electron transport and reaction properties acquired within the last few years. Many questions of a more fundamental nature concerning the physico-chemical properties of excess electrons in liquids are unanswered. We are continuing to provide answers to these questions particularly in the areas of the effect of high electric fields on the electron-solvent and electron-solute interactions. Our current sutdies, which all use the pulse conductivity technique that we developed to measure electron attachment rates (1) and electron mobilities are described in the following. Unless noted otherwise, these studies are done in collaboration with Dr. W.F. Schmidt of the Hahn-Meitner Institute in West Berlin.

I) Simulation of Transport Tones in Cellular Water

Our work on the energy dependence of electron attachment reactions in atomic liquids (32,33) is now being extended to

the molecular liquids neopentane, TMS and CH_4 which were chosen for this study because all three solvents exhibit electron "heating" effects at a critical field, E_c, above which the electron mobility decreases. The E_c values of these liquids are neopentane, 15 kV/cm (6), TMS, 10 kV/cm (7) and CH_4, 1.5 kV/cm. SF_6 was chosen as the electron acceptor since this molecule has an extremely energy dependent electron attachment cross section in both the gas (29) and liquid (32,33) phases which peaks at thermal energy.

Preliminary results in neopentane-SF_6 solutions indicate no change in the electron attachment rate constant of $2.5 \times 10^{14} M^{-1} s^{-1}$ at electric fields ranging from 5 to 50 kV/cm. A decrease of $\sim$50 percent, from 2.6 to $1.8 \times 10^{14} M^{-1} s^{-1}$, was measured in TMS-$SF_6$ solutions over the same range of fields. Preliminary experiments in CH_4-SF_6 solutions from 0.5 to 5 kV/cm indicate a similar energy dependence of the attachment rate constant; however, all of these measurements will be repeated in a newly designed ion chamber having larger electrodes and a greater inter-electrode distance. This modification will reduce electron loss to the electrodes which is the electron decay process that competes with electron attachment to SF_6.

Studies of the field dependence of electron attachment to SF_6 in C_2H_6 and C_3H_8 were also initiated. The transport properties of these liquids over the temperature range of -140 to -50°C contrast markedly with those of neopentane, TMS and CH_4 which is reflected in μ_e ranging from $<10^{-3}$ to

$\sim$1 cm^2/volt sec. These low mobilities are due to polaron-like transport of the electrons in C_2H_6 and C_3H_8 with the electron hopping between solvent traps (34-36). In earlier studies we had determined the electron mean jump distance and the jumping frequency from the high field transport properties of these liquids.

The jumping frequency increases at high fields due to distortion of the electron-solvent potential well which results in a field dependent increases in μ_e. We hypothesized that this increased jumping frequency would be reflected in an increased attachment rate of electrons under high field conditions.

The attachment rates measured at -78°C in C_2H_6 and C_3H_8 are consistent with this hypothesis. The rate constants increased proportionately with the field enhanced mobility at electric fields ranging from 50-100 kV/cm. This study is now being extended to lower temperatures at which the jumping frequency of C_2H_6 decreases but that of C_3H_8 is unchanged.

In addition to these electron attachment studies in liquids having electron mobilities <1 and >70 cm^2/volt sec, we are preparing to study the electron attachment process in the intermediate electron mobility region of 5-70 cm^2/volt sec. We have measured the electron mobility in isooctane-TMS mixtures and found values of 18 cm^2/volt sec at 23 mole percent and 63 cm^2/volt sec at 83 mole percent isooctane in TMS. The electron mobilities of intermediate mixtures will be determined along with the high field electron transport

behavior. When these transport properties are established for the mixtures, the low and high field electron attachment rates will be studied in order to close the gap of knowledge that now exists in this intermediate electron mobility region (3).

II) Electron Transport in Pure Liquids

a) Conventional Pulse Conductivity

We have begun another study designed to bridge the gap between low and high mobility electron transport processes. Our earlier studies of liquid ethane covered a temperature range from -160 to -60°C with corresponding electron mobilities ranging from $\sim 10^{-3}$ to 1 cm^2/volt sec (34-36). We are now able to extend this study through the critical temperature of liquid ethane at 32.1°C by using an ion chamber designed by W. Doldissen of the Hahn-Meitner Institute, West Berlin, who is also collaborating in this study. The 50Ω stainless steel ion chamber is capable of withstanding internal pressures of >50 atm. and electric fields of 180 kV/cm have been applied without breakdown.

An electron mobility of ~ 40 cm^2/volt sec was measured in liquid C_2H_6 at 20°C and the mobility was found to decrease at fields >50 kV/cm which indicates that the transition from polaron to delocalized electron transport had occurred. The electron mobility increased monotonically through the critical temperature which is in contrast to the electron mobility maxima observed in other liquids near the critical temperature (30,31). Measurements at temperatures from -40 to +40°C may indicate at what mobility the electron transport mechanism

changes. Electron attachment studies in liquid C_2H_6 at these same temperatures are planned.

The final study of electron mobilities in liquids using conventional pulse conductivity techniques that we are conducting is to determine if an isotope effect exists for electron transport in liquids. Among the liquid hydrocarbons that we have studied, CH_4 is the easiest to purify and has well defined electron transport characteristics (31,36); consequently, we have chosen the deuterated analog for our isotope effect study. We are now in the process of preparing CD_4 of sufficient purity to measure the electron transport properties in this liquid at fields ranging from 10^2-10^4 kV/cm.

b) Picosecond Pulse Conductivity

The application of picosecond, ps, sampling techniques to pulsed conductivity measurements by Dr. G. Beck of the Hahn-Meitner Institute, West Berlin has opened up a new time regime for studying electron transport and attachment processes in non-polar liquids. We are collaborating with Dr. Beck in this pioneering area and already have directly observed processes that appear to conflict with existing theory which of necessity has been based on post nanosecond measurements.

The train of 50 ps fine structure pulses that make up the "3" nanosecond electron pulses from the Hahn-Meitner Linac is illustrated in Figure 3A. The dots represent the 5 ps sampling times; the time between each train of pulses in 20 milliseconds.

The growth in the conductivity signal produced by irradiating TMS in an ion chamber with an applied field of 10 kV/cm by bremstrahlung produced from the electron pulses in 3A impinging on a lead target is shown in Figure 3B. The conductivity signal grows with each irradiation pulse and no significant decay by attachment, recombination or drift to the electrode occurs. This is the behavior expected for electrons in a pure liquid at this dose and with this electric field. However, the slow rise in the growth of the conductivity signal after each pulse is not consistent with existing theory.

An expansion of this slow growth by the sampling computer is shown in Figure 3C where the rise time from 10-90 percent of the growth is seen to require ∿160 ps. This is significantly longer than the rise time of the cell and sampling system which is ∿30 ps as demonstrated by the spikes observed from the same cell and shown in Figure 4. Attempts to increase this slow growth by using cells having electrodes of different areas and separated at different distances did not decrease the rise time of the conductivity signal.

We tentatively offer two explanations for the observed slow growth. One is the production of meta-stable super-excited states which have lifetimes ranging from 10-150 ps and which upon ionizing eject electrons which then contribute to the conductivity signal. To test this, benzene, a classical scavenger of excited states, was added at a concentration of ∿0.5M to the TMS. No change in the conductivity growth was observed which suggests that delayed ionization is not the

answer. However, other excited state scavengers will be used before rejecting this hypothesis.

A second explanation is that the electron thermalization time in TMS may be as long as 160 ps. Hot electrons which have a more random motion than thermal electrons and would, therefore, have a lower drift velocity and contribute less to the conductivity. Upon thermalizing at later times, the electrons would make their full contribution to the conductivity signal. This hypothesis is in contradiction to existing thermalization theory which states that electrons in liquids reach thermal energy within 0.1 ps after the ionizing event. This hypothesis is now being tested by adding solutes which should be efficient electron thermalizers to the TMS.

A second set of observations shown in Figures 4A and B also conflict with existing theory. Irradiating TMS directly with the fine structure electron pulses rather than bremstrahlung to increase the dose-rate produced the large, fast current spikes on the normal conductivity signal. The spikes were found to be dose, field and polarity dependent and were reproduced in different samples of TMS.

In Figure 4A with _10 kV/cm applied, no spike was observed until the dose (and conductivity signal) had accumulated to the level shown (scaling factors to give the conductivity currents are not available at this time). A second spike was observed when the fourth fine structure pulse raised the conductivity signal to the same level. At 5,000 kVcm, no spikes were observed and at higher fields breakdown occurred.

The effect of the field polarity on the spikes is shown in Figure 4B. Under identical conditions as A except for the reversed polarity, the spike was observed after the second pulse irradiated the TMS (same ordinate scaling factor in A and B). At -5kV/cm, no spikes were observed.

We tentatively propose that the spikes are a pre-breakdown phenomenon which may involve an electron avalanching effect that extinguishes in a few ps. Such effects have been proposed in breakdown theory but have not been observed at these low fields. We were able to resolve these spikes only because of the resolution of the ps sampling technique; the same spikes would have been integrated by conventional electronics to an almost negligible perturbation of the conductivity signal.

These preliminary experiments demonstrate that a new field of knowledge is available for exploration by ps conductivity sampling techniques. This technique should be valuable in improving our understanding of electronic transport and reaction processes both in radiation chemistry and radiation biology.

c) Liquid-filled Radiation Detectors

The recent development of a variety of liquid argon-filled radiation detectors indicate that these detectors have advantages of increased sensitivity and spatial resolution compared to conventional gas-filled or solid-state detectors. These advantages, however, are offset by the cryogenic problems associated with liquid argon.

Our studies of TMS, tetramethyl tin and, currently tetramethyl lead at high electric fields suggest that these liquids would be ideal for an ambient liquid-filled detector. An ion chamber having an alpha ray source at one electrode has been constructed and the response characteristics of the chamber filled with TMS are being determined.

FIGURE LEGENDS

Figure 1. Enhancement ratio as a function of log kT for the substituted nitrobenzene compounds listed in Table IV.

Figure 2. Dependence of electron mobility on solvent composition in isooctane-tetramethylsilene mixture at 20°C.

Figure 3. (A) Train of five 50 picosecond fine structure pulses in the Hahn-Meitner Institute Linac electron pulse.
(B) Growth of conductivity signal from a parallel-plate ion chamber filled with TMS at 20°C and with an applied field of 10 kV/cm following irradiation by bremstrahlung from a lead target irradiated with pulses shown in A.
(C) Details of conductivity growth in first two pulses of B.

Figure 4. (A) Observance of "prebreakdown" conductivity pulses at third and fourth pulses of TMS irradiated by electron fine structure pulses with applied field of 10 kV/cm.
(B) Same conditions as A except negative polarity of applied field. Note that "prebreakdown" occurs at second pulse (lower dose than third pulse of A) and that rise-time of spike is ∿50 picoseconds.

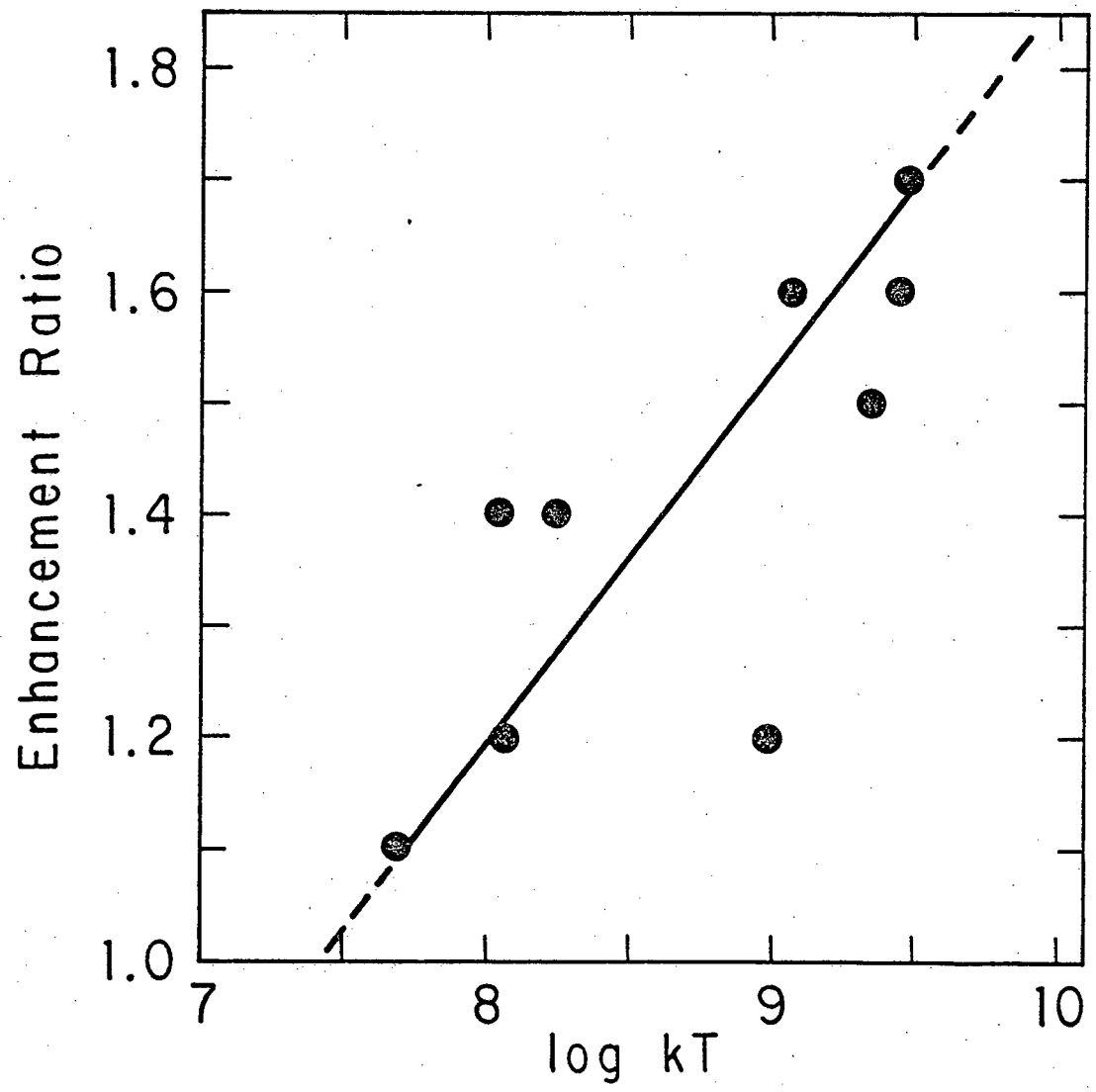

Figure 1

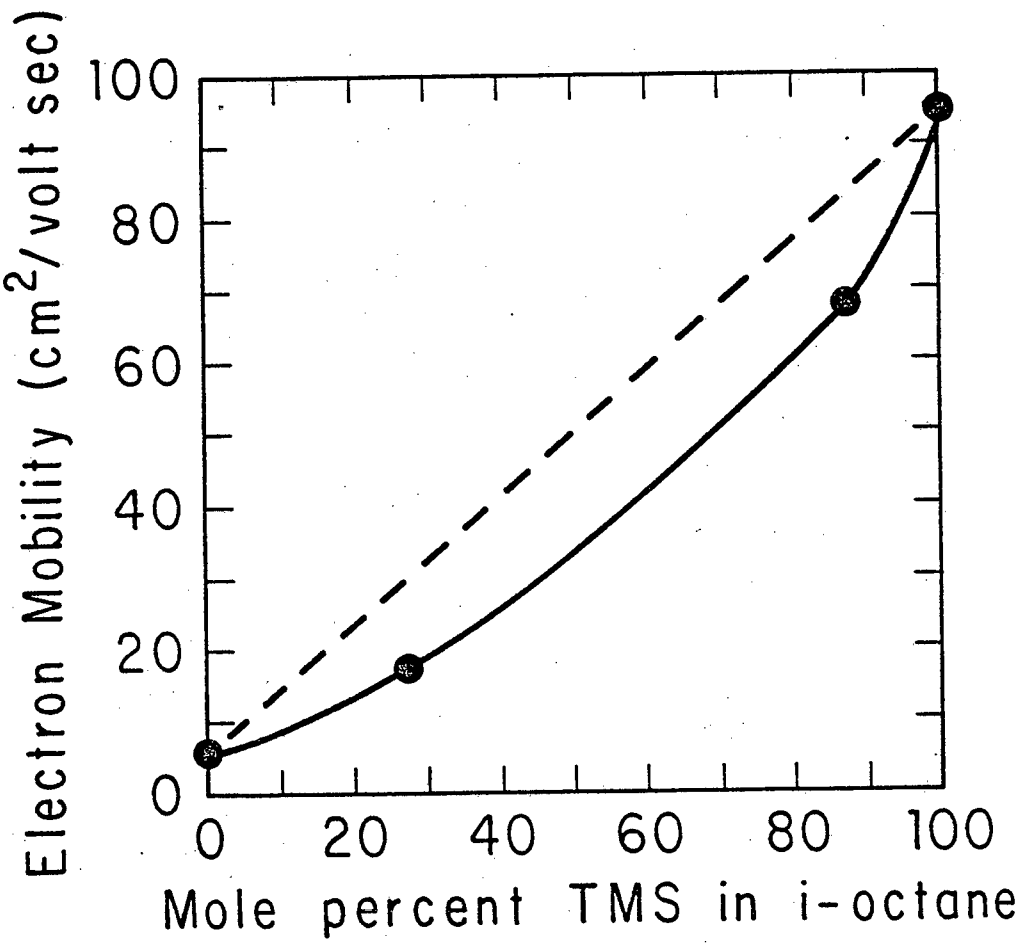
Electron Mobility (cm^2/volt sec)
100
80
60
40
20
0
0
20
40
60
80
100
Mole percent TMS in i-octane

Figure 2

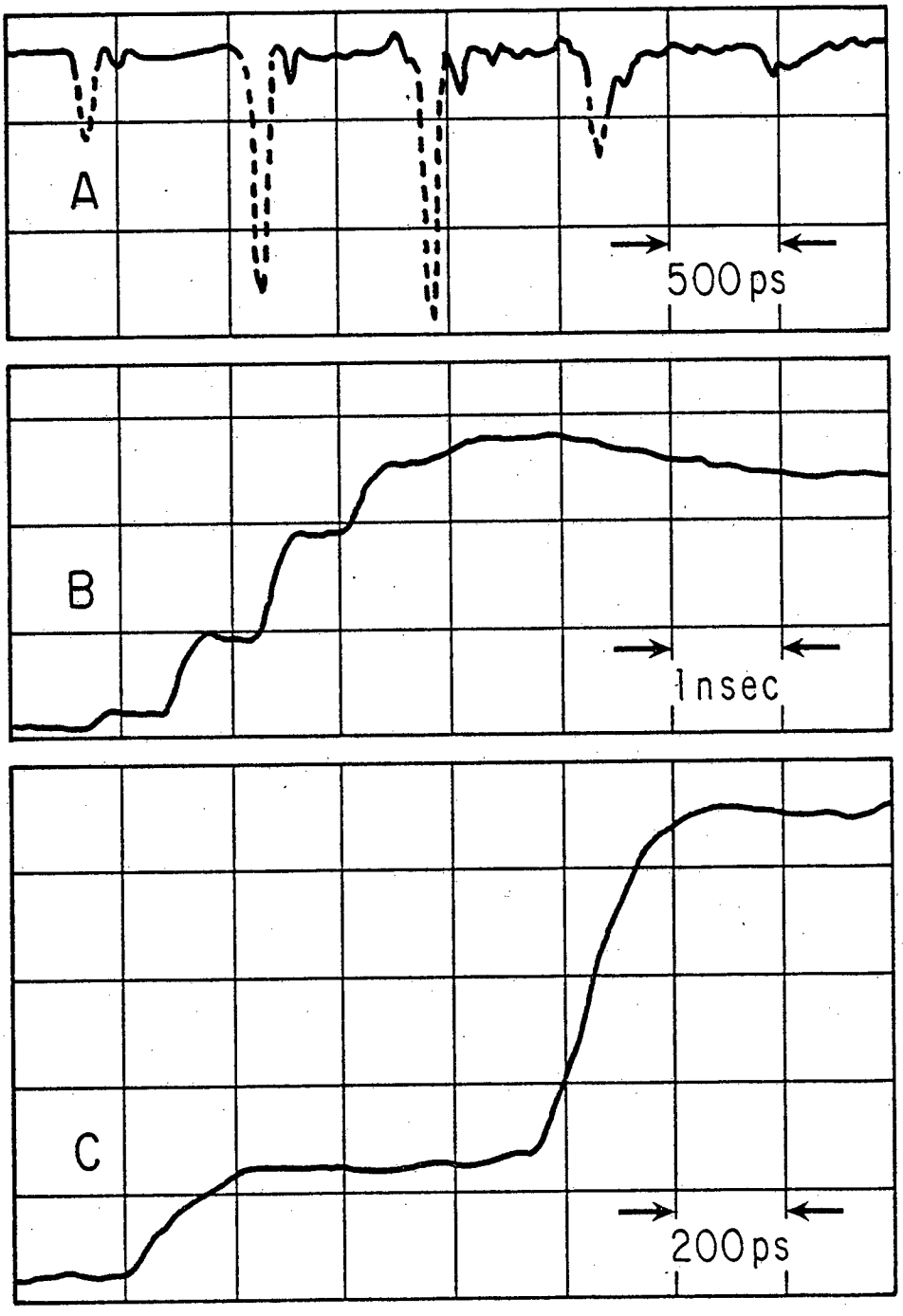

Figure 3

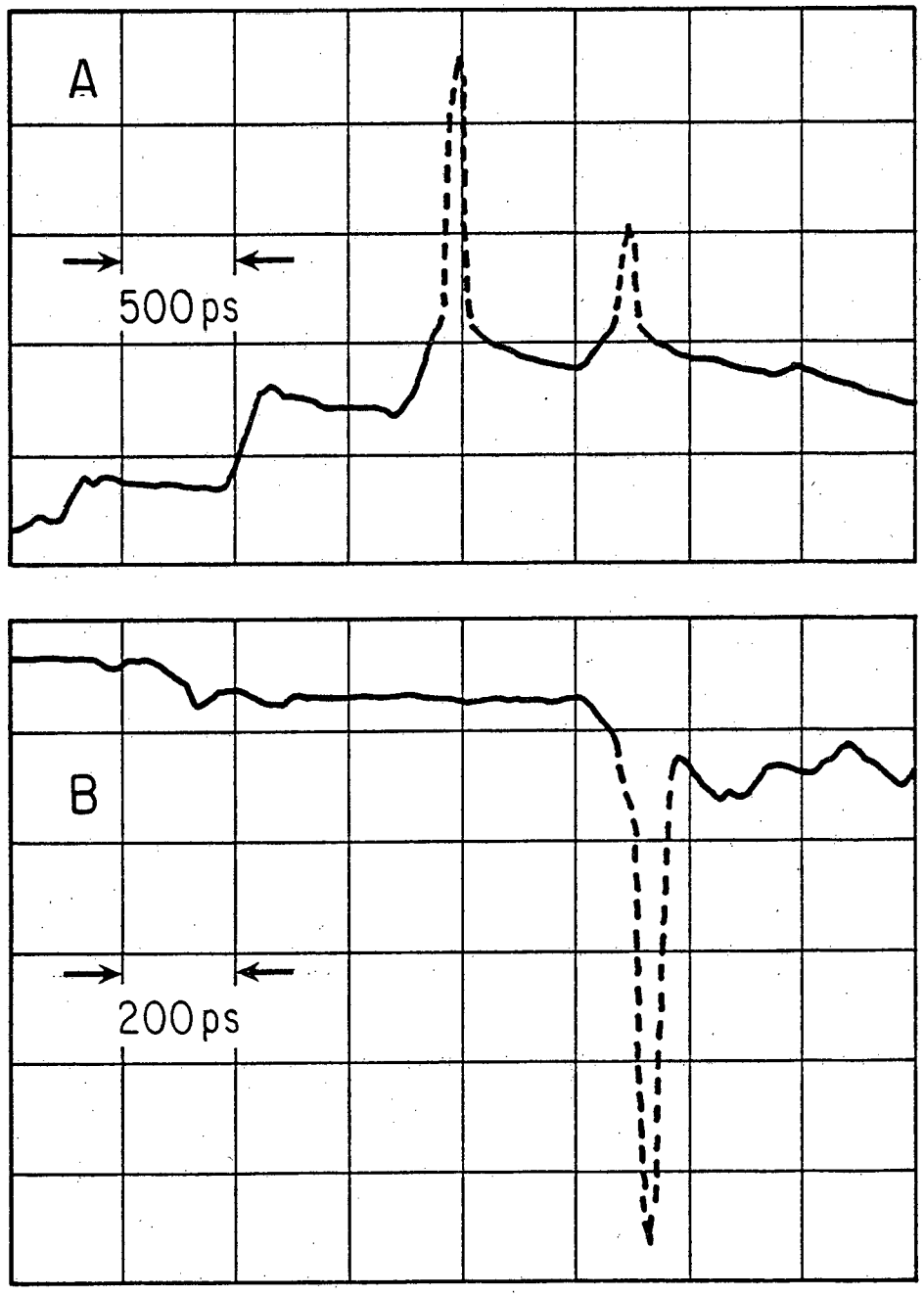

Figure 4

REFERENCES

1. G. Bakale, E.C. Gregg, and R.D. McCreary, "Decay of Quasifree Electrons in Pulse-Irradiated Liquid Hydrocarbons", J. Chem. Phys. 57, 4246 (1972).

2. A.O. Allen, T.E. Gangwer and R.A. Holroyd, "Chemical Reaction Rates of Quasifree Electrons in Non-Polar Liquids, II", J. Phys. Chem. 79, 25 (1975).

3. G. Bakale, U. Sowada and W.F. Schmidt, "Electron Attachment to Sulfur Hexafluoride in Nonpolar Liquids", J. Phys. Chem. 79, 3041 (1975).

4. R.E. Weston, Jr. and H.A. Schwarz, "Reactions in Solution", Chapt. 6, Chem. Kinetics, Prentice Hall, Englewood Cliffs, N.J. (1972).

5. J.P. Dodelet and G.R. Freeman, "Mobilities and Ranges of Electrons in Liquids: Effect of Molecular Structure in C_5-C_{12} Alkanes", Can. J. Chem. 50, 2667 (1972).

6. J.W. Hunt, "Early Events in Radiation Chemistry", in Advances in Radiation Chemistry (M. Burton and J.L. Magee, Eds.) 5, pp. 185-315, John Wiley and Sons, New York (1976).

7. K.Y. Lam and J.W. Hunt, "Picosecond Pulse Radiolysis, VI. Fast Electron Reactions in Concentrated Solutions of Scavengers in Water and Alcohols", Int. J. Radiat. Phys. Chem. 7, 317 (1975).

8. E.C. Gregg, R.D. McCreary and G. Bakale, "Effect of Biologically Important Solutes on Charge-Carrier Decay Processes in n-Hexane", presented at the Fifth International Congress of Rad. Res. Seattle, Washington, July 13-20 (1974).

9. J. McCann and B.N. Ames, "Detection of Carcinogens as Mutagens in the Salmonella/microsome Test: Assay of 300 Chemicals: Discussion", Proc. Nat. Acad. Sci. USA 73, 950 (1976).

10. B.A. Bridges, "Short Term Screening Tests for Carcinogens", Nature 261, 195 (1976).

11. R.A. Holroyd, T.E. Gangwer, and A.O. Allen, "Chemical Reaction Rates of Quasi-Free Electrons in Non-Polar Liquids. The Equilibrium $CO_2 + e^- \rightleftharpoons CO_2^-$", Chem. Phys. Lett. 31, 520 (1975).

12. J.M. Warman, M.P. DeHaas, E. Zador, and A. Hummel, "Concerning the Equilibrium e^- + Biophenyl $\rightleftharpoons$ Biphenyl$^-$ in Liquid Tetramethylsilane", Chem. Phys. Lett. 35, 383 (1975).

REFERENCES (Cont.)

13. D.Dee and M.E. Baur, "Charge and Excitation Migration in DNA Chains", J. Chem. Phys. 60, 541 (1974).

14. D. Vasilescu, "Some Electrical Properties of Nucleic Acids and Components in Physico-Chemical Properties of Nucleic Acids", J. Duchesne, ed. 31-66, Academic Press, New York (1973).

15. H. Meier, Organic Semi-Conductors, Ch. 11, "Photobiological and General Technical Processes", pp. 435-437, Verlag Chemie, Weinheim, W. Germany (1974).

16. J.M. Warman and A. Hummel, private communication

17. G. Bakale and E.C. Gregg, "Conjecture on the Role of Dry Electrons in Direct Radiosensitization", to be presented at the 8th L.H. Gray Conference, Cambridge, England, Sept. 5-9, 1977 and published in the British J. of Cancer (1978). See appended Abstract.

18. 1976 Annual Report to ERDA

19. J.P. Johnson, D.L. McCorkle, L.G. Christophorou, and J.G. Carter, "Long-lived Parent Negative Ions Formed via Nuclear-excited Feshbach Resonances. Part 4. Systematic Study of NO_2-containing Benzene Derivatives", J. Chem. Soc. Farad. Trans. II, 71, 1742 (1975).

20. A.G. Walton and J. Blackwell, "Biopolymers, Ch.2, Conformation", pp. 39-40, Academic Press, Inc., New York (1973).

21. J.H. Fendler, "Interactions and Reactions in Reversed Micellar Systems", Acc. Chem. Res. 9, 153 (1976).

22. W. Arnold, "Paths of Electrons in Photosynthesis", Proc. Natl. Acad. Sci., USA 73, 4502 (1976).

23. M. Tomkiewicz and G.A. Corker, "Chlorophyll Sensitized Charge Separation in Phospholipid Vesicles", Photochem. Photobiol. 22, 249 (1975).

24. M. Calvin, "Photosynthesis as Resource for Energy and Materials", Photochem. Photobiol. 23, 425 (1976).

25. F.M. Menger, J.A. Donahue and R.F. Williams, "Catalysis in Water Pools", J. Am. Chem. Soc. 95, 286 (1973).

26. J.B. Peri, "The State of Solution of Aerosol OT in Nonaqueous Solvents", J. Cell. Interf. Sci. 29, 6 (1969).

27. A.J. Frank, M. Gratzel, A. Henglein and E. Janata, "The Influence of the Interface Potential on the Reactions of

Hydrated Electrons and Neutral Radicals with Acceptors in Micelles", Ber. Bunsenges. Physik. Chem. 80, 547 (1976).

28. A.J. Frank, M. Gratzel, A. Henglein and E. Janata, "Electron Transfer Reactions of Singlet and Triplet Pyrene in Micelles with Various Radical Anions in Aqueous Solutions", Ber Bunsenges. Physik. Chem. 80, 294 (1976).

29. L.G. Christophorou, D.L. McCorkle and J.G. Carter, "Cross Sections for Electron Attachment Resonances Peaking at Subthermal Energies", J. Chem. Phys. 54, 253 (1971).

30. J.P. Dodelet and G.R. Freeman, "Electron Mobioities in Alkanes through the Liquid and Critical Region", Can. J. Chem. 55, 2264 (1977).

31. J.M.L. Engels and A.J.M. Van Kimmenada, "The Mobility of Excess Electrons in Liquid Methane", Chem. Phys. Letters, 42, 250 (1976).

32. U. Sowada, G. Bakale, K. Yoshino and W.F. Schmidt, "Electric Field Effect on Electron Capture by SF_6 in Liquid Argon and Xenon", Chem. Phys. Lett. 34, 466 (1975).

33. G. Bakale, U. Sowada and W.F. Schmidt, "The Effect of an Electric Field on the Electron Attachment to SF_6, N_2O, and O_2 in Liquid Argon and Xenon," J. Phys. Chem. 80, 2556 (1976).

34. G. Bakale, U. Sowada and W.F. Schmidt, "Electron Transport in Low Mobility Liquid Hydrocarbons", 1974 Annual Report, Conference on Electrical Insulation and Dielectric Phenomena, 41, National Academy of Sciences, Washington, D.C. (1975).

35. W.F. Schmidt, G. Bakale and U. Sowada, "Excess Electrons and Positive Charge-Carriers in Liquid Ethane", J. Chem. Phys. 61, 5775 (1974).

36. G. Bakale, W. Tauchert and W.F. Schmidt, "Electron Transport in Mixtures of Liquid Methane and Ethane", J. Chem. Phys. 63, 4470 (1975).

APPENDICES

I) Papers delivered and published in the period Dec. 1976 through Nov. 1977.

II) Abstract of paper to be delivered at the 8th L.H. Gray Conference, Sept. 1977.

III) Preprint of paper accepted for publication in J. Chem. Phys. (1977).

PAPERS PRESENTED AND PUBLISHED DURING THE
LAST YEAR PERTINENT TO
"IONIZATION IN LIQUIDS"

1. G. Bakale, U. Sowada and W.F. Schmidt, "Electron Transport in Liquid Tetramethylsilane", High Energy Chemistry, 10, 323 (1976).

2. G. Bakale, U. Sowada and W.F. Schmidt, "Effect of an Electric Field on Electron Attachment to SF_6, N_2O and O_2 in Liquid Argon and Xenon", J. Phys. Chem. 80, 2556 (1976).

3. U. Sowada, G. Bakale and W.F. Schmidt, "Dependence of Electron Mobility on Electric Field Strength in Nonpolar Liquids", High Energy Chemistry 10, 290 (1977) translated from Khimiya Vysokikh Energii 10, 323 (1976).

4. G. Bakale, E.C. Gregg and R.D. McCreary, "Structural Effects on the Electron Attachment Rates of Nitroaromatic Molecules and Implications to Radiation Sensitization", presented at the Radiation Research Society Meeting, San Francisco, CA, June 27, (1976) and to be submitted for publication in Radiation Research (1977).

5. G. Bakale, U. Sowada and W.F. Schmidt, "Negative Ion-Molecule Reactions in Liquid Argon Following Electrons Capture by N_2O", presented at the Electrons in Fluids Conference, Banff, Alberta, Canada, September 5 (1976) and published in Can. J. Chem. 55, 2220-2224 (1977).

6. U. Sowada, W.F. Schmidt and G. Bakale, "Influence of Non-electronegative Molecules on the Low-Field Electron Mobility in Liquid Rare Gases", presented at Electrons in Fluids Conference, Banff, Alberta, Canada, September 5 (1976) and published in Can. J. Chem. 55, 1885-1889 (1977).

7. G. Bakale, "Electron Attachment to Substituted Nitrobenzenes", presented at The Reactor Institute, Delft, The Netherlands, May 31, 1977.

8. G. Bakale, E.C. Gregg and R.D. McCreary, "Electron Attachment to Nitro Compounds in Liquid Cyclohexane", to be published in J. Chem. Phys., (1977).

9. G. Bakale and E.C. Gregg, "Conjecture on the Role of Dry Electrons in Direct Radiosensitization", to be presented at the Eighth L.H. Gray Memorial Conference, Cambridge, England, Sept. 5-9, 1977 and to be published in the British Journal of Cancer (1978).

Project 2

THE MECHANISM OF DNA REPLICATION AND THE EFFECT OF IONIZING RADIATION ON THIS PROCESS

PROGRESS REPORT

covering the period of September 1, 1976 - August 31, 1977

Principal Investigator: E.N. Brewer, Ph.D.

Department of Radiology

Case Western Reserve University
Cleveland, Ohio

September 1, 1977

Prepared for

THE U.S. ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION
UNDER CONTRACT NO. EY-76-S-02-2486.A001

Project No. 2

The Mechanism of DNA Replication and the Effect of Ionizing Radiation on this Process

Abstract

A simplified growth medium has been devised for cultivation of Physarum polycephalum. With this medium, growth rates are higher, and intermitotic times shorter and more reproducible than were obtained previously. A stimulatory factor for DNA synthesis in isolated nuclei of Physarum has been found in aqueous extracts of this organism. The active substance is heat-stable, non-dialyzable, insoluble in hot ethanol, and destroyed by ashing. Rejoining of γ-radiation-induced double-strand breaks has been observed in nuclei isolated from Physarum. Strand-break rejoining in vitro requires Mg^{++} and dextran, but not EGTA, ATP, or deoxyribonucleoside triphosphates. Significantly less rejoining occurs in nuclei isolated from S-phase, as compared to G_2-phase plasmodia.

E.N. Brewer, Ph.D. Principal Investigator, Project No. 2

Time devoted to project since the beginning of the current term of the agreement: 80%

Time expected to be devoted during the remainder of the current term: 80%

The Mechanism of DNA Replication in Physarum polycephalum and the Effect of Ionizing Radiation on this Process

E.N. Brewer

For some time the principal goals of this laboratory have been to determine the biochemical requirements for eukaryotic DNA replication and for the repair of DNA damaged by ionizing radiation. During the current contract period we have made further progress toward the achievement of both of these objectives:

1) a stimulatory factor for DNA synthesis in isolated nuclei of Physarum polycephalum has been partially purified; 2) rejoining of radiation-induced double-strand breaks in nuclei isolated from this organism has been demonstrated. In addition, we have devised a simplified version of the standard growth medium used to cultivate Physarum. Using this improved medium, the plasmodial growth rate is higher and the nuclear division cycle shorter and more reproducible.

A. A simplified growth medium for Physarum polycephalum.

In order to determine the possible effects of Ca^{++} on DNA strand break rejoining following irradiation of intact plasmodia of Physarum (cf. Proposed Research, 1976-1977), attempts were made to cultivate the organism on nutrient media containing various levels of Ca^{++}. Although Ca^{++} has been reported to be necessary for growth of Physarum (Daniel and Rusch, Develop. Biol., 25, 47-59, 1961), we found instead that cultures grew at least as vigorously in the absence, as in presence, of Ca^{++} salts. Consequently, the requirements for each of the other components of the Daniel-Rusch medium were investigated. It was found that added $PO_4^{=}$, Mn^{++}, Fe^{++} and Zn^{++} (beyond the levels present in the yeast extract and tryptone components of this medium) were also unnecessary for growth of microplasmodia in shaken culture. Indeed, with the nutrient medium which we have adopted for routine use in this labora-

tory (Brewer and Prior, Physarum Newsletter, 8, 45, 1976), growth rates are somewhat higher, and intermitotic times shorter and more reproducible than had been obtained previously. The studies reported below were carried out using cultures of Physarum maintained at 26.5°C on this simplified growth medium.

B. Biochemical requirements for DNA synthesis in isolated nuclei of Physarum polycephalum

We have shown previously that maximal DNA synthesis in homogenates or isolated nuclei of Physarum requires the presence of Mg^{++}, EGTA, dextran, ATP, and all four deoxyribonucleoside triphosphates (Brewer and Ting, J. Cell. Physiol., 86, 459-470, 1975, COO-78-312; Brewer, Biochim. Biophys. Acta, 402, 363-371, 1975, COO-78-334). Many other exogenous substances have been tested for their ability to stimulate DNA synthesis in such nuclear preparations. None has yet been found to stimulate synthesis significantly in vitro with the exception of protamine. However, the latter substance appears to prevent damage to nuclei during homogenization of plasmodia, rather than to stimulate the rate or extent of DNA synthesis per se (Brewer, Annual Report, 1975-6, COO-2486-365).

More recently, we have found that a substance present in the post-nuclear supernatant fraction is able to stimulate synthesis in nuclei which have been washed several times with the plasmodial homogenizing medium, but not in homogenates or unwashed nuclei (Fig. 1). It seems likely, therefore, that a substantial portion of this substance is extracted from nuclear preparations by repeated washing.

The stimulatory factor is also present in heated (100°C for 5 min) water extracts of either S- or G_2-phase plasmodia. From such heated extracts the active principle can be recovered by lyophilization. This activity is non-dialyzable, insoluble in hot ethanol, and is destroyed by ashing (Table I).

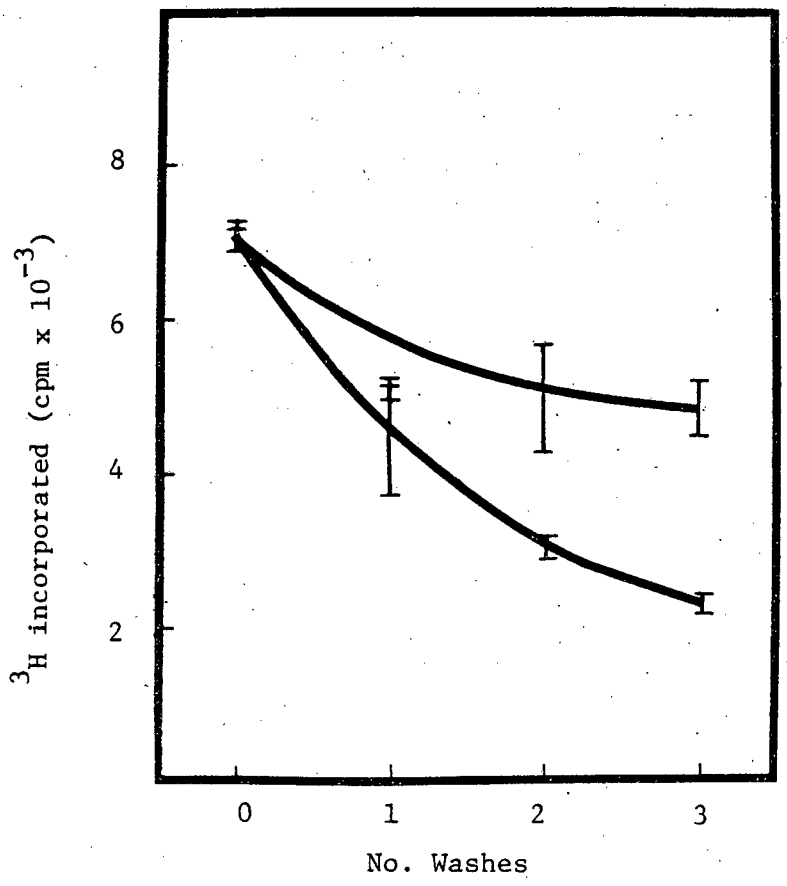

Fig. 1 Stimulation of DNA synthesis in nuclei isolated from Physarum. Nuclei were isolated during early S phase, washed with homogenizing medium as shown on the abscissa, and resuspended in the original post-nuclear supernatant fraction. Incorporation of [^{3}H]dATP during a 120-min incubation period was determined as described previously (see text).

Table I. Properties of the stimulatory factor for DNA synthesis in washed nuclei.

Expt.	Preparation	cpm ± av. dev.
1	H_2O (heated)	3600 ± 210
	H_2O extract (heated)	4568 ± 540
	dial. heated H_2O ext.	4558 ± 252
2	H_2O (heated)	2544 ± 63
	H_2O extract (heated)	5253 ± 375
	lyoph. heated H_2O ext.	5583 ± 93
	residue after hot ethanol extn.	4980 ± 120
3	H_2O (heated)	3620 ± 234
	H_2O extract (heated)	4454 ± 315
	residual ash	3682 ± 412

Concentrated H_2O extracts were prepared by homogenizing plasmodia with 2 volumes of H_2O, centrifuging at 10,000 x g for 10 min, heating at 100° for 5 min, and centrifuging at 10,000 x g for 10 min. Heated extracts (or heated H_2O) were then diluted 9:1 with homogenizing medium and assayed for stimulation of DNA synthesis in washed nuclei as described previously (see text). Dried residues were taken up in heated H_2O prior to array.

Thus, the evidence obtained so far suggests that this endogenous stimulatory factor is a relatively high-molecular-weight substance. Attempts to further purify the active material are in progess (see Proposed Research).

In a similar study, nuclei were extracted with the plasmodial homogenizing medium containing Kyro EOB (a non-ionic detergent), 0.5M NaCl, or 0.3 M magnesium acetate, all of which greatly reduce nuclear DNA-synthesizing activity. In these cases, however, the DNA-synthesizing activities of the residual nuclei could not be restored by recombination with the corresponding nuclear extracts (Table II).

C. Joining of DNA strand breaks in nuclei isolated from Physarum

For the past several years we have attempted, unsuccessfully, to demonstrate rejoining, in vitro, of DNA "single-strand breaks" (assayed by alkaline sucrose density gradient sedimentation) produced by γ-irradiation of intact plasmodia of Physarum (see Annual Reports 1972-1976). Recently, however, we have found that substantial rejoining of double-strand breaks in isolated nuclei can be demonstrated by neutral sucrose density gradient centrifugation analysis (Fig. 2). Unlike the requirements for DNA replication in nuclear preparations (see section B, above), joining of double-strand breaks does not require addition of EGTA, ATP, or the deoxyribonucleoside triphosphates to the cell-free system. Dextran and Mg^{++}, however, are required for maximal joining activity. Little rejoining is seen after 1 hr of incubation in vitro, but rejoining appears to be maximal after 2 hr, and no further shift in the sedimentation profile can be seen after a 4-hr incubation period (Table III).

Rejoining of double-strand breaks has been demonstrated previously in intact plasmodia of Physarum (Brewer and Nygaard, Nature New Biol., 229, 108-110, 1972, COO-78-267). In that communication we showed that plasmodia irradiated

Table II. Effect of various nuclear extracts on DNA synthesis in extracted nuclei of Physarum.

Expt.	Preparation	Resusp. Medium	cpm ± av. dev.
1	Unextracted nuclei	Regular homogenizing medium	6913 ± 158
	Nuclei extracted with 0.1% Kyro EOB	" + dil. Kyro	1669 ± 30
	" "	" + dil. Kyro extract	1575 ± 120
2	Unextracted nuclei	Regular homogenizing medium	8631 ± 16
	Nuclei extracted with 0.5 M NaCl	" + dil. NaCl	1063 ± 16
	" "	" + dil. NaCl extract	1002 ± 8
3	Unextracted nuclei	Regular homogenizing medium	6263 ± 650
	Nuclei extracted with 0.3 M magnesium acetate	" + dil. Mg^{++}	2262 ± 79
	" "	" + dil. Mg^{++} extract	2597 ± 188

Nuclei isolated from Physarum by the standard method were extracted with 0.1% Kyro EOB, 0.5 M NaCl, or 0.3 M magnesium acetate, resuspended in the appropriate medium and incubated with [^{3}H]dATP as usual (Brewer, Biochim. Biophys. Acta, 402, 363-371, 1975, COO-78-334). (The detergent or salts were in the plasmodial homogenizing medium). Extracts were prepared by extracting 10 X the number of nuclei assayed. The concentrated extracts were diluted 9:1 with homogenizing medium in order to reduce the detergent or salt contents to levels which have relatively little inhibitory effect. The magnesium acetate extract was dialyzed against regular homogenizing medium in order to eliminate excess Mg^{++}. In all cases, the appropriate control media were similarly treated.

Fig. 2. Rejoining of γ-radiation-induced DNA strand breaks in nuclei isolated from Physarum. Plasmodia were prelabeled, from the time of fusion, with [^{3}H] or [^{14}C]thymidine (cf. Brewer and Nygaard, Nature New Biol., 229, 108-110, 1972, COO-78-267). During the G_2 period preceding metaphase III, or 30 min after MIII, halves of the [^{3}H]-labeled plasmodium were exposed to 90 kR ^{60}Co γ-radiation. Nuclei were then isolated, resuspended in the standard homogenizing medium and incubated for 0 or 2 hr at 35°C. An unirradiated [^{14}C]-labeled plasmodium was treated identically. After incubation, [^{3}H]- and [^{14}C]-labeled nuclei were mixed, and neutral sucrose density gradient sedimentation profiles determined as described previously (Brewer, J. Mol. Biol., 68, 401-412, 1972, COO-78-260; Brewer, Biochim. Biophys. Acta, 402, 363-371, 1975, COO-78-334). Direction of sedimentation is from right to left.

FIG. 2

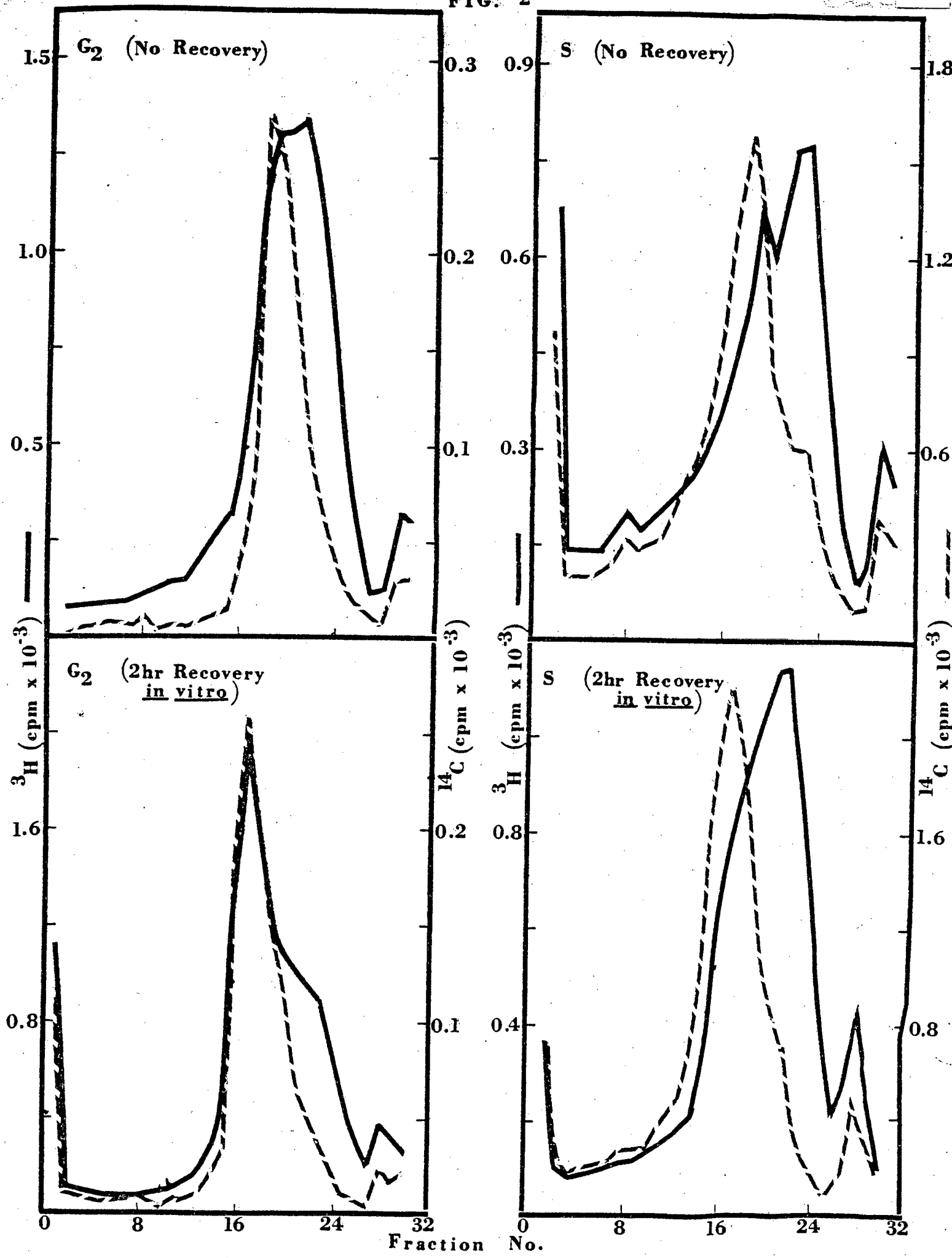
G2 (No Recovery)
S (No Recovery)
G2 (2hr Recovery in vitro)
S (2hr Recovery in vitro)
3H (cpm x 10-3)
14C (cpm x 10-3)
Fraction No.
0
8
16
24
32

Table III. Requirements for rejoining of DNA strand breaks *in vitro*.

System	Rejoining
Complete, 2 hr incubation	+++
" , - ATP, -dNTP's,-EGTA	+++
" , " -dextran	-
" , " $-Mg^{++}$	+
Complete, 0 hr incubation	-
" , 1 hr incubation	+
" , 4 hr incubation	+++

Plasmodia were irradiated during G_2 (90 kR), and nuclei were isolated and incubated as described in Fig. 2. Joining equivalent to that shown in Fig. 2 for nuclei isolated from G_2-phase plasmodia incubated for 2 hr *in vitro* is indicated by +++. A slight but detectable increase in the sedimentation coefficient of the irradiated DNA is indicated by +. The absence of a detectable shift in the sedimentation profile is indicated by -.

during the S period were less able to complete the repair of strand breaks (single- or double-) than were those irradiated during G_2. We hypothesized at that time that radiation damage to replicating DNA might become "fixed" by fork movement past damaged sites. It is of some interest, therefore, that the present data indicate that double-strand breaks produced by irradiation of G_2-phase plasmodia are also repaired to a considerably greater extent, in vitro, than are those of S-phase plasmodia, under conditions (-ATP, -dNTP's) which support very little DNA synthesis (Fig. 2). The present data suggest that this cell cycle-related difference in the ability to effect the rejoining of strand breaks may be an intrinsic property of the nuclei themselves, rather than a result of damage fixation brought about by replication of damaged DNA sites.

The apparent absence of repair of "single-strand breaks" under conditions which support considerable double-strand break rejoining, raises a question concerning the nature of the strand breaks manifested in alkaline sucrose density gradients. Our data suggest, indeed, that the majority of such breaks may represent secondary strand scissions at alkali-labile regions in irradiated DNA as opposed to frank strand breaks resulting directly from irradiation (see also Kay and Ward, Radiat. Res. 69, 185-193, 1977; Matsudaira et al., Biochim. Biophys. Acta, 476, 97-107, 1977). It should be noted that there appears to be greater initial damage to DNA irradiated during the S phase, as compared to the G_2 phase (Fig. 1). Since this finding differs somewhat from that reported earlier (Brewer and Nygaard, Nature New Biol., 229, 108-110, 1972, COO-78-267), in which study the γ-radiation was delivered at a much higher dose rate (ca. 8 kR/min) than available at present (ca. 3 kR/min), it seems likely that some of the DNA damage observed in the current study may be repaired during the exposure period. In addition, a substantial portion (perhaps 20%) of the DNA of S-phase plasmodia undergoes

replication during the 30 min exposure to ^{60}Co. These complications make interpretation of the results somewhat more difficult. It is hoped, however, that this problem can be alleviated in the near future by recharging of the ^{60}Co γ-radiation source.

APPENDIX

A SIMPLIFIED GROWTH MEDIUM FOR PHYSARUM POLYCEPHALUM

E.N. Brewer and Amanda Prior

We have devised a simplified version of the Daniel and Rusch (J. Gen. Microbiol., 25, 47-59, 1961) growth medium. One liter of the modified medium contains 10 g tryptone, 3 g yeast extract, 9 g dextrose, 3.6 g citric acid, and 0.5 g $MgSO_4 \cdot 7H_2O$; pH is brought to 4.6 with 30% KOH, and sterile hematin solution added to the sterile medium as usual. Microplasmodial growth rate is about 10% faster for the modified medium, and interdivision times are reduced from 9 to about 8 h, and are less variable, for stationary macroplasmodia.

[Physarum Newsletter, 8, 45 (1976)]