COO-4746-3

MASTER

IONIZATION IN LIQUIDS

PROGRESS REPORT

covering the period of September 1, 1977 - April 30, 1981

Principal Investigator: G. Bakale, Ph.D.
Co-Principal Investigator: E.C. Gregg, Ph.D.

Department of Radiology
Case Western Reserve University
Cleveland, Ohio

December 19, 1980

Prepared For
THE U.S. DEPARTMENT OF ENERGY
UNDER CONTRACT NO. DEAS0278EV04746

DISCLAIMER

TABLE OF CONTENTS

PERSONNEL

Principal Investigator:	G. Bakale, Ph.D., Assistant Professor 80% effort, 60% funding
Co-Principal Investigator:	E.C. Gregg, Ph.D., Professor 10% effort, no funding
Equipment Development:	W.H. Voelker, Ph.D., Assistant Professor 10% effort, no funding
Accelerator Operator:	R.D. McCreary 100% effort, 100% funding
Research Associate:	M. Isildar, Research Associate 2 months @ 100% effort and funding
Research Assistant:	T. Wasi-Gilbert, Jr. Research Assistant 5 months @ 100% effort and funding

OTHER FEDERAL SUPPORT OF PERSONNEL

G. Bakale, Ph.D.	DHEW Grant CA 19283	40% salary
Earle C. Gregg, Ph.D.	DHEW Grant CA 15901 DHEW Grant CA 19283	(10% effort & salary) (10% effort & salary)
W.H. Voelker, Ph.D.	DHEW Grant CA 19283	25% salary

GRANT LISTING

DHEW CA 19283:	"Core Support for Radiation Oncology Research" Joint Principal Investigators: E.C. Gregg, O.F. Nygaard and R.J. Alfidi
DHEW CA 15901:	"Mutants and Altered Radioresponse of Cells and Tumors" E.C. Gregg, Principal Investigator

SUMMARY

Significant progress has been made in clarifying the transport and reaction properties of quasifree electrons in model nonpolar liquids and biological systems during this triennial reporting period. Our experiments and those of others indicate that quasifree electrons simulate the behavior of unsolvated or dry electrons in aqueous media including the special case of biological systems.

We developed a model of direct radiosensitization based on dry charge-carriers having an extended lifetime in the sheath of structured water that surrounds polar biomolecules. This model utilized results of picosecond studies in which the pre-solvation lifetimes of dry electrons were shown to increase with an increase in the rotational times of solvent molecules. Our measurements of the quasifree electrons attachment rate constants, k_e's, of radiosensitizers and biomolecules also contributed to the development of this model which was found to be consistent with several of the observed radiation-induced DNA-damaging properties of electron affinic radiosensitizers.

Concurrently with the development of this model, an increasing number of radiosensitizers were found to be carcinogenic. Consequently, we measured the k_e's of known carcinogens and noncarcinogens and found that 37 of 42 carcinogens attached quasifree electrons at diffusion-controlled rates, whereas the k_e's of 30 of 34 noncarcinogens were significantly less. We also found the quasifree electron attachment rates of various types of cigarette smoke to be correlated with the cigarette tar content.

These results indicated that k_e measurements could serve as a valuable carcinogen-screening test and implied that unsolvated electrons play a role in the initiating step of the carcinogenesis mechanism. To explore the k_e-carcinogenicity correlation further, we undertook a study of quasifree electron attachment to the water pools of reversed micelles that we found could be used to encapsulate microsomal enzymes that activate procarcinogens to their ultimate reactive forms. In these reversed-micelle studies, which were conducted in the picosecond time regime, we also controlled the degree of structuredness of the water pools which determines the k_e of the reversed micellar system.

Another approach to controlling the microenvironment of quasifree electrons in biological systems was done in studies of radiation-induced damage to DNA in concentrated DNA solutions. The high concentration of DNA induces more structure into the solutions than that occurring in typical in vitro experiments, and this structural enhancement extends the lifetime of unsolvated charge-carriers. A second technique of determining the DNA-damaging effects of radiolytically produced charge-carriers is through our recently initiated studies of synergistic mutagenesis in bacteria that we simultaneously exposed to ionizing radiation and electrophilic chemical carcinogens.

A study that also involved quasifree electron reaction properties of a solute of biological interest was the attachment-detachment equilibrium of nicotine in hexane solutions. In this work, both the kinetics and the therodynamics of electron reactions were studied. An analogous study of the attachment-detachment electron equilibrium of p-difluorobenzene in hexane solutions provided a model system for the nicotine equilibrium. A study of electron attachment to CO_2 in cyclohexane indicated that an attachment-detachment equilibrium also occurs in this system at room temperature.

Studies of a more fundamental nature included high-field mobility measurements of electrons in ethane under a wide range of conditions in which the transition from localized to extended-state electron transport was observed. In another high-field study, the first observations of field-dependent k_e's of localized electrons were made in solutions of SF_6 in liquid ethane and propane.

PROGRESS REPORT

I. Introduction

Our study of phenomena related to ionization in liquids during this reporting period, September 1, 1977 to April 30, 1981, has focussed intensively upon the transport and reaction properties of quasifree, or excess, electrons in nonpolar liquids. The unique properties and ubiquitous nature of this most fundamental reducing species has drawn the attention of chemists and physicists to radiation chemistry, the field in which quasifree electron studies were initiated about ten years ago. Through our research program we have attempted to acquaint radiobiologists with this species so that knowledge which has been gained of the physico-chemical properties of quasifree electrons might be applied to the vast number of biologically related electron-transfer reactions. We recognize that an enormous gap exists between electron reactions in nonpolar liquids and electron reactions in vivo, but we shall demonstrate how this gap has been reduced in the past few years and how we have contributed to this reduction.

Our studies of quasifree electrons can be divided into two main areas, studies of model liquids and of biologically related systems. We shall first report our studies in the former area which we sub-divide into quasifree electron transport studies and quasifree electron reactions with solutes. Among the solutes studied are membrane-mimetic reversed micelles, which

form a link to our biologically oriented work. We shall then conclude with a description of our studies of the role of quasifree electrons in direct radiosensitzation and of the significance of the quasifree electron attachment rate of a solute serving as an indicator of that solute's carcinogenic propeties.

II. Electron Transport in Model Liquids

A key factor in understanding the electron transport properties of liquids is a detailed knowledge of the electron transport mechanism. We have contributed significantly to this area through our high-field studies of the electron drift velocity, v_d.

The v_d of a thermal delocalized electron that drifts in an applied field E has a mobility u_e given by:

$$u_e = v_d/E \qquad (I)$$

i.e., u_e remains constant as v_d increases proportionally with E. This condition applies to fields at which a thermal equilibrium is maintained between the electron and the solvent molecules which act as absorbers of the excess energy that the electron gains from the field between collisions. As the field is increased, however, a critical field E_c is reached above which the electron cannot transfer all of the energy gained from the field to the solvent molecules and the electron energy

increases. This excess energy increases the randomness of the electron motion and is reflected in v_d increasing less than proportionally with E and approximately with $E^{1/2}$; i.e., at $E > E_c$, $v_d \propto E^{1/2}$.

This hot-electron effect was first observed in a condensed medium by Schockley in his studies of electron transport in semiconductors (1), and we made the first observations of this effect in molecular liquids. The liquids that we studied included methane (2,3), neopentane (4), tetramethylsilane or TMS (5,6), and solutions of methane and ethane (7,8). In addition to the $E^{1/2}$ dependence of v_d at $E > E_c$ characterizing extended-state electron transport, all of these liquids have low-field u_e's exceeding 50 cm^2/Vs, and this high value of u_e serves as a second characteristic of non-localized electron transport.

In contrast to this type of behavior, we have found that excess electrons that are localized by the solvent exhibit a much different behavior. In addition to having low-field u_e's that are significantly less than those of delocalized electrons (generally < 1 cm^2/Vs), the high field v_d of localized electrons increases approximately with E^2 at $E > E_c$. The actual field dependence is given more rigorously by

$$\frac{u_e(E)}{u_e(0)} = \frac{2\ k\ T}{\lambda\ eE} \sinh\left(\frac{\lambda e\ E}{2\ kT}\right) \qquad (II)$$

where $u_e(E)$ and $u_e(0)$ are the u_e's above and below E_c, respectively, k is the Boltzmann constant, T is the absolute temeprature, λ is the distance between localization sites and e is unit electronic charge. The field-enhanced u_e may be visualized as an enhancement of the escape probability of the electron from its localization site when the field is sufficiently increased to distort the trap which allows more facile electron escape. This field-assisted detrapping model was developed by Bagley (9) and exhibits the same field dependence of u_e at the fields we have studied as the small polaron model of Reik (10) and Efros (11).

We have found the Bagley model to be consistent with the field-dependent u_e's that we observed in liquid ethane (4,12,13), propane (6,12) and n-pentane (6). A more rigorous treatment of the field dependence of the u_e's in these liquids which involves electron transport over barriers of fluctuating heights has been proposed (14), but this model was recently shown to yield the same result as the original Bagley model (15). It appears that all of the models considered thus far suffer from one-dimensional analysis of a three-dimensional problem (15,16). This will be discussed in more detail in the following description of our recently completed study of ethane (16-18, see Appendix).

This introduction to our ethane studies stresses that

electron transport in nonpolar liquids occurs through either a localized or a delocalized mechanism. Our earlier studies of ethane (4,12,13) and methane-ethane mixtures (7,8) indicated that ethane provided us with the ideal medium in which to study the transition from localized electron transport at low temperatures to delocalized transport at higher temperatures. We have reported the results of this study (16-18 and Appendix) and shall now briefly summarize this work.

The pulse-conductivity technique previously used in all of our measurements of v_d vs E was again used and was described in detail in Reference (3). Measurements near the critical temperature, T_c, of 305.33°K necessitated our using an ion chamber capable of withstanding pressures in excess of 100 atmospheres. Electron attachment to impurities at the higher temperatures was also a problem which was circumvented by reducing our drift-time measurements to ~15 nsec, and this required that the ion-chamber have 50-Ω impedance [see Fig. 2 of Reference (16) in Appendix]. The electrode area-gap ratio that was required for the 50-Ω impedance cell necessitated the use of a guard ring to reduce fringe-field effects. With this 50-Ω high pressure ion-chamber and with a 500 MHz pulse amplifier, the response time of the entire measurement system was ~1 nsec.

The inter-dependence of temperature and density effects on v_e complicates straightforward analysis of the results, and isochoric and isobaric measurements were conducted to delineate

these effects on u_e. The transition from localized to extended-state transport in the liquid ethane was found to occur between 240 and 260°K where the values of u_e were 3.5 and 5.5 cm^2/Vs, respectively. In this temperature range the dependence of v_d on E changed from E^2 (localized) to $E^{1/2}$ (extended state). The liquid density in this transition region ranges from 12.5 - 15.5 moles/l, which is in good agreement with the theoretical prediction by Kimura and Fueki of the transport transition to occur in this density range (19). This result is also consistent with our earlier studies of methane-ethane mixtures which indicated that the localized to extended-state transition occurred in an equimolar mixture of ethane and methane where u_e is ~ 5 cm^2/Vs at 111°K and where the ethane partial density is 10.5 mole/l (8). Thus, it appears that methane has a negligible effect on electron localization.

From the dependence of v_d on E at $E > E_c$ in the extended-state transport region, we found that the mean fractional energy loss, f, by an electron in an inelastic collision with ethane is $\sim 10^{-3}$, which is significantly greater than $f = \sim 4 \times 10^{-4}$ in electron-methane collisions which we had previously determined (5). This is in agreement with the intuitive expectation that ethane is a better absorber of electron energy than methane.

The same high-field data can also be compared with liquids in which electron transport is known to be in an extended state.

In argon, methane, neopentane and TMS, u_e passes through a maximum that is 2-10 times the u_e at T_c; however, we observed only a much less pronounced maximum in the u_e of ethane at temperatures approaching T_c. We conclude that additional electron scattering mechanisms occur with non-spherical ethane molecules that do not occur in liquids of atoms or spherical molecules where Lekner's theory is more applicable to the observed mobility maxima (20).

We also made a careful study of the temperature dependence of u_e at densities near the critical density, ρ_c, which is 6.8 mole/l for ethane. A shallow u_e minimum was observed at ρ_c which is another effect predicted by Lekner, who with Bishop ascribed the minima as arising from electrons being localized by long-wavelength density fluctuations of the fluid near the critical point (21). We used the theory of Lekner and Bishop to calculate a critical point u_e of 57 cm^2/Vs in ethane, which is in reasonable agreement with our measured value for the u_e minimum of ~ 40 cm^2/Vs. The agreement between theory and experiment for ethane may be fortuitous, however, since an order of magnitude discrepancy was found for the u_e minimum at the critical point of argon (21).

We also found a strong temperature dependence of u_e in ethane at densities near ρ_c which also is consistent with extended-state electron transport. The marked dependence on T at densities near ρ_c arises from the strong temperature dependence

of the compressibility of the fluid at these densities, which again is consistent with Lekner theory (20).

We conclude this discussion of electron transport in ethane by restating that a full description of our results is given in the appended Reference (16). In the same study we also measured positive ion mobilities which were found to follow Walden's rule; i.e., the product of the ion mobility and the liquid viscosity was constant.

III. Electron Attachment in Model Liquids

A. Field-Dependent Electron Attachment

We have illustrated in the preceding Section that our studies of the field dependence of the electron mobility have been valuable in elucidating the details of the electron transport mechanism in several nonpolar liquids. Our studies of the field dependence of electron attachment to several solutes in liquid rare gases analogously provided new knowledge of the mechanism of delocalized electron attachment in liquids (22,23). With this experience in field-dependent electron transport and attachment, we undertook a study of field-dependent attachment of localized electrons. For this study, we chose the electron-accepting solute that has been most thoroughly studied in the gas and liquid phases; viz., SF_6. We also chose as the solvents for this study the two liquids in which we had most intensively studied the field-dependent v_e's, namely, ethane and propane. A full description of this recently completed study is appended, pp. A-74 to A-91, and we shall now briefly summarize this work.

The pulse-conductivity technique used to measure the electron attachment rate constants or k_e's was again used (24,25). At each temperature at which k_e's were measured, inter-electrode distances and SF_6 concentrations were chosen to optimize electron decay by attachment to the solute while minimizing electron losses by attachment to impurities, by

neutralization at the anode, and by recombination with ions. Despite our efforts to maximize the attachment process, corrections for electron decay by the three other decay modes were required. The estimated error in the reported values of k_e is ± 25 percent.

Several typical examples of the enhancement of the k_e by E when E exceeds E_c are shown in Figure 1, p. A-89 of Appendix. The increase in k_e at $E > E_c$ reflects the field-enhanced increase in v_e that we had observed earlier in liquid ethane (4,12,13) and propane (12) and the diffusion-controlled attachment of localized electrons by SF_6 that we had reported in ethane, propane and other "low-mobility" solvents (25).

The simplified form of the Smoluchowski equation which is applicable to localized electron attachment to SF_6 (see pp. A-80 and A-81 of the Appendix for details) is given by:

$$k_e = 4\pi R D_e \qquad \text{(III)}$$

where R is the effective encounter radius between the electron and SF_6 and D_e is the diffusion coefficient of the localized electrons. Values of D_e may be obtained from the Nernst-Einstein equation:

$$D_e = \mu_e kT/e \qquad \text{(IV)}$$

The proportional dependence of k_e on D_e expected from Eq. (III) is shown in Figures 2 and 3, pp. A-90 and A-91 of Appendix, for ethane and propane, respectively, for which D_e was evaluated from our earlier measurements of u_e in this liquids. The slopes of the plots of k_e vs D_e for both ethane and propane yield R = 14.5 A.

Before discussing the dynamics of the electron-SF_6 interaction that leads to the large observed value of R, we note that the proportional dependence of k_e on D_e implies that the ratio eD_e/u_e is apparently field-independent in liquid ethane and propane. This conclusion contrasts with the results of Shibamura et al who recently found that this ratio was field-dependent in liquid argon (26). We surmise that the observed field dependence in the latter study is related to extended-state electron transport and inefficient momentum transfer from the delocalized electrons to the solvent atoms in liquid argon, whereas the localized electrons in liquid ethane and propane remain at thermal energies at even the highest fields studied, which was $\sim$250 kV/cm.

We return now to the observed value of R = 14.5 A in liquid ethane and propane. The hard core radius of SF_6 is only 2.5 Å, which suggests that long range electron-SF_6 interactions must be considered to account for the remaining difference of 12 Å. Baird has considered electron-SF_6 interactions in several solvents and also noted large values of R (27). If we follow

Baird's approach to treating the electron-induced dipole interaction but assume no screening of the interaction other than through the dielectric constant, ε, of ethane, the distance r_α at which the electron-induced dipole interaction balances the thermal energy is given by:

$$r_\alpha = (\alpha e^2/2\varepsilon kT)^{1/4} \qquad (V)$$

At T = 195°K where ε = 1.75 (27) and $\alpha(SF_6)$ = 6.5 $\mathring{A}$ (28), r_α is found to be 7 $\mathring{A}$. This represents a maximum of the electrostatic interaction since screening effects by the solvent were ignored, but r_α(max) is seen to account for only about half of the observed R. Baird suggested that the discrepancy was due to breakdown of Eq. (V) at distances approaching R and/or electron tunneling to SF_6 at R (27). Although we agree with these possible explanations, we offer a third alternative.

In our discussion of the field-dependent u_e's of localized electrons in Section II, we described the field-enhanced u_e's as resulting from distortion of the localization barriers by the applied field with the enhancement being given by Eq. (II). Funabashi and Rao's modification of Eq. (II) to include fluctuating barrier heights (14) and a more recent treatment of a similar problem by Rao et al (29) in which disorder was introduced into the electron-hopping mechanism both led to an inter-localization distance λ of 7.5 - 10 $\mathring{A}$. We

suggest that R exceeds r_α because electrons "instantaneously" hop to the electron-induced dipole capture radius r_α of 7 Å from a distribution of distances of which λ is the upper limit; i.e., $R = r_\alpha + \bar{\lambda}$, where $\bar{\lambda}$ is the mean hopping distance of ~5Å. Thus, R would be expected to be 12 ± 3 Å, which is in reasonable agreement with our observed value of 14.5 Å. This explanation of the large vlaue of R in the electron attachment mechanism is also consistent with that proposed by Yakovlev *et al* for electron scavenging in normal and cyclohexane (30).

Our field-dependent u_e studies have received considerable attention from theoreticians (14,27,29,31-34), and we anticipate that our field-dependent k_e studies will recieve even more attention. One of the fundamental problems of radiation chemical physics which has evolved from Onsager's classical electron escape-probability study (35) is to accurately describe the lifetime distribution of ion-pairs. One of the most successful approaches to treating this problem is to obtain information about the ion-pair lifetime distribution from experimentally measured electron and/or ion scavenging yields by using an inverse Laplace transformation [for a review of this subject, see Reference (36)]. Information on the short-time distribution of ion-pair lifetimes requires that scavenger concentrations > 0.1 M be used, and at this concentration the average inter-scavenger distance is ~25 Å. Therefore, any charge scavenging that occurs deep within the Onsager critical radius

can be expected to be field-dependent, but until now such field-dependent scavenging has been ignored, although attempts to consider the effects of field-dependent electron mobilities on the escape probability have been made (31-34).

Our field-dependent electron mobility and attachment studies also have practical implications to dielectric breakdown phenomena. A number of studies have indicated that quasifree electrons are involved in the initiation of the breakdown process in dielectric liquids (37-39), and with the new knowledge that our high-field electron transport and reaction studies provide, a better understanding of the role of quasifree electrons in dielectric breakdown is anticipated.

B. Electron Attachment to Polar Solutes

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 k_e's of a series of 39 nitrocompounds having dipole moments that ranged from 0.5 to ~7 Debye. A full description of this work has been published (40) and is included in the Appendix, pp. A-5 to A-11. We shall now briefly summarize this work and discuss our subsequent studies of electron-dipole interactions.

As in the field-dependent electron-attachment study, the k_e's were again measured using a pulsed-conductivity technique, but the experimental error was considerably less (±10%) than in the field-dependent k_e study (±25%) because measurement conditions could be optimized to reduce electron losses by recombination and by drift to the anode. Values of k_e in cyclohexane at 293°K ranged from 3-7 x 10^{12} $M^{-1}s^{-1}$ for the nitroaromatic compounds studied. Several nitro compounds were studied in both normal and cyclohexane and the measured k_e's were found to be about threefold greater in cyclohexane. This threefold difference also applies to the u_e's in the two solvents [u_e(n-hexane, 294°K) = 0.07 cm^2/Vs cf. u_e(c-hexane, 294°K) = 0.22 cm^2/Vs, Reference (41)], which suggests that the k_e's are diffusion-limited. Thus, we again begin our discussion of the results with the Smoluchowski equation, Eq. (III) on p. 13.

Incorporation of both electron-induced dipole and electron-dipole interaction for electron attachment to polar solutes modifies Eq. (III) by increasing R. On pp. A-8 to A-9 of the Appendix we derive the modified R to be:

$$R^{-1} = 1/2 \left(\frac{\pi}{a}\right)^{1/2} \mathrm{erf}(a^{1/2}/r_\alpha^*) \qquad \text{(VI)}$$

where $a = e\,\mu_{eff}/\varepsilon kT$ and μ_{eff} is the effective dipole moment of the solute and r_α^* is the distance at which the electron-induced dipole interaction energy is equal to kT. The effective dipole

moment, μ_{eff} , is dependent upon the electron-dipole orientation angle θ and is given by:

$$\mu_{eff} = \mu \cos \theta \qquad \text{(VII)}$$

where μ is the solute dipole moment.

Values of k_e and μ_{eff} were calculated for solute dipole moments ranging from 0.5 to 7 Debye and agreed within the experimental error with the measured rate constants for 36 of 39 monosubstituted nitrobenzenes and for several poly-substituted nitrobenzenes. From studies of the latter we concluded 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.

We predicted from this study that the electron-dipole interaction should influence k_e more strongly in "low-mobility" solvents such as cyclohexane than in liquids such as TMS where u_e is 95 cm^2/Vs (41). This prediction is based on the dipole of the solute requiring a finite time to interact with the electron and on this interaction time increasing if the electron diffuses more slowly by the dipole. Thus, localized electrons in cyclohexane have a greater probability of a maximum interaction with a polar solute than do delocalized electrons in TMS.

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 o- and p-DNB attachment rates in cyclohexane.

These k_e's are the highest attachment rates observed in a liquid at room temperature [cf. $k_e(SF_6) = 2.1 \times 10^{14} M^{-1} s^{-1}$ (41)] which suggests that these reactions are diffusion-controlled. Using Eq. (IV) to evaluate $D_e = 2.4\ cm^2/s$ from $u_e = 95\ cm^2/Vs$ and substituting this value into Eq. (III) yields R = 2.8 Å, which is approximatley the hard-core radius of the solute. Thus, it appears that no electron-dipole interaction occurs in electrons that attach to polar solutes in TMS.

Cyclohexane and TMS represent limiting cases of solvent effects on electron-dipole interactions. We are currently studying electron attachment to nitroaromatic solutes in a solvent in which u_e is intermediate between these extremes; viz, in isooctane for which $u_e = 5.3\ cm^2/Vs$ (41). The preliminary k_e's of several nitroaromatic solutes that we have recently measured in isooctane at room temperature are listed in Table I, p. 21, with the dipole moment of each solute. We can draw no conclusions as yet from these cursory results, but it should be apparent that this study will provide information on the degree of electron localization in isooctane. This is particulary significant since the "quasi-localized" electron transport

Table I. Dipole moments and electron attachment rate constants of solutes measured in cyclohexane and isooctane at 20°C.

Solute	isomer	μ, Debye	Solvent, $k_e \times 10^{-12}$, $M^{-1}s^{-1}$ c-hexane	i-octane
Carbon Tetrachloride		0	3.0	7.7
Dinitrobenzene	o-	6.1	6.7	53
	p-	0.5	2.8	7.3
Nitrobenzonitrile	o-	6.2	6.6	41
	p-	0.7	4.0	34
Fluoronitrobenzene	o-	5.0	5.7	45
	m-	3.6	5.0	29
	p-	2.6	3.4	15
Nitrotoluene	o-	3.7	5.0	35
	m-	4.2	5.8	41
	p-	4.4	5.3	31
Nitroaniline	o-	4.1	4.9	10
N,N-dimethylnitroaniline	o-	4.2	5.6	22
	p-	6.9	6.7	40

mechanism in solvents in which u_e ranges from 0.1 - 10 cm^2/Vs is the least poorly understood mobility regime (42). This is reflected in deviations from proportionality of plots of k_e vs D_e in this mobility range (43,44) and our high-field transport studies indicating that v_d appears to show both an E^2 and an $E^{1/2}$ field dependence (4,8,12).

C. Electron Attachment-Detachment Equilibria

Prior to this reporting period, we made the first direct observations of an ion-molecule detachment reaction in the liquid phase (45). The detachment process that we observed may be viewed as being related to Reaction (2) in the equilibrium

$$e^- + A \underset{2}{\overset{1}{\rightleftharpoons}} A^- \qquad (1,2)$$

in that both reactions liberate a quasifree electron from an anion. Following our detachment study, we initiated studies of electron attachment-detachment equilibria in nonpolar liquids, a process that also has been investigated by other groups (46-48). A complete report (49) of our study of electron attachment to and detachment from para-difluorobenzene, p-DFB, in normal and cyclohexane is appended, pp. A-25 to A-29. We shall now briefly describe this work and our subsequent studies of electron attachment-detachment equilibria in other systems.

As in our other studies, pulse conductivity was used to produce quasifree electrons in normal and cyclohexane solutions and to monitor their reaction with p-DFB. Values of k_1 and k_2 for the attachment and detachment reactions, respectively, in the hexanes are listed in Table I of Reference (49), p. A-26, at temperatures from 5-28°C. Also included in Table I are values of the equilibrium constant, K, which is the ratio k_1/k_2, and the temperature dependence of K is plotted in Figure 2, p. A-27. The

bimolecular attachment reaction k_1 values are listed in units of $M^{-1}s^{-1}$, whereas the unimolecular detachment reaction has dimensions of s^{-1}; consequently K is expressed in M^{-1}.

The attachment reactions are diffusion-limited and, therefore, exhibit temperature dependences that are proportional to D_e. In contrast, the detachment reactions are extremely temperature sensitive and are characterized by large activation energies (19.2 and 21.6 kcal/mol for n- and c-hexane, respectively) and by large pre-exponential factors. Activation energies of this magnitude would usually preclude the observance of these reactions in the sub-microsecond time regime, but the large pre-exponential factors compensate for this energy requirement.

Treating this reaction according to transition-state theory leads to activation entropies of 34 and 44 cal/ mol in n- and c-hexane, respectively, which indicates that polarization of solvent molecules around the anions plays a significant role in the detachment reactions. We used the Born equation to evaluate a standard free energy of polarization of $\sim$-1eV or -23 kcal/mol for both solvents. Other thermodynamic parameters for the equilibria were also evaluated (see pp. A-27 and A-28) and were combined to yield an electron affinity of -0.34 eV for p-DFB. This result is intermediate between other measurements of the electron affinity of p-DFB that range from -0.54 (50,51) to +0.18 (52) eV. We used this result and the electron affinities

of other solutes in several solvents (47,48) to demonstrate that the free energy of reaction is linearly dependent on the solute electron affinity.

Another solute that we have studied during this Report period is nicotine, which also temporarily attaches electrons in n- and c-hexane at temperatures from 5-30°C. Values of k_1, k_2 and $K = k_1/k_2$ for electron-nicotine attachment-detachment equilibria in n- and c-hexane are presented in Table II. The enthalpies of reaction derived from plots of these data are -28.9 kcal/mol in c-hexane and -32.0 kcal/mole in n-hexane.

The values of k_1 at room temperature in both solvents suggest that the electron attachment reaction is diffusion-controlled; however, the negative temperature dependence of k_1 indicates otherwise. Another characteristic of the nicotine equilibrium in these solvents is a secondary attachment reaction denoted by k_3 which exhibits a positive temperature dependence. The two electron attachment rate constants k_1 and k_3 suggest that nicotine attaches electrons at two sites with the two sites having significantly different k_e's. Since nicotine may be viewed as being composed of two molecules, pyridine and N-methylpyrrolidine, we studied electron attachment to these solutes to determine if these two molecules that compose nicotine attach electrons at rates k_1 and k_3. An attachment-detachment equilibrium was observed for pyridine in cyclohexane and the temperature dependence of K for pyridine

Table II. Values of k_1, k_2, k_3 and K as a function of temperature for nicotine in:

A. Cyclohexane

T (°K)	$10^3/T$ ($°K^{-1}$)	$k_1 \times 10^{-12}$ ($M^{-1}sec^{-1}$)	$k_2 \times 10^{-6}$ (sec^{-1})	$K_{eq} \times 10^{-6}$ (M^{-1})	$k_3 \times 10^{-11}$ ($M^{-1}sec^{-1}$)
285	3.51	2.58	0.8	3.23	5.8
287	3.48	2.55	1.1	2.32	6.0
289	3.46	2.46	1.3	1.90	5.0
291	3.44	2.26	1.9	1.19	5.0
294	3.41	2.31	3.0	0.77	4.4
295	3.39	2.21	3.9	0.57	5.2
297	3.37	2.59	6.8	0.38	4.0
299	3.34	2.40	8.5	0.29	3.5
301	3.32	2.14	10.2	0.21	4.5
303	3.30	2.19	12.8	0.19	3.6

B. n-Hexane

283	3.53	1.45	0.60	2.41	3.3
285	3.52	1.16	0.70	1.66	3.3
287	3.48	0.97	0.85	1.15	2.0
289	3.46	1.04	1.5	0.70	3.4
291	3.44	1.03	2.0	0.51	2.9
293	3.41	0.92	2.6	0.36	3.6
295	3.39	0.66	3.2	0.21	3.2
297	3.37	0.66	3.85	0.17	2.9
299	3.34	0.67	6.5	0.10	2.8

is compared with the K for nicotine in Figure 1, p. 28. The enthalpy of reaction is -26. kcal/mol, which is slightly less than the value found for nicotine. A significant k_3 was also observed for electron attachment to pyridine in c-hexane. Electron attachment to N-methylpyrrolidine was negligible; an upper limit of k_1 of this solute in c-hexane is $10^{10} M^{-1} s^{-1}$. Thus, two attachment sites for electrons to nicotine does not appear to explain our results since pyridine itself appears to attach electrons at two markedly different attachment rates.

We are currently attempting to determine the significance of the k_3's of nicotine and pyridine. Our cursory results are less reproducible than those of p-DFB which suggests that an electron-attaching impurity may contribute to the observed k_3's. Consequently, we plan to repeat our measurements for nicotine and pyridine in the hexanes with freshly purified solutes; this is discussed in more detail in the 1981-82 Research Proposal.

Another solute that we have found to attach and detach electrons in cyclohexane is CO_2. This molecule is of obvious biological significance and was found by Baxendale et al (53) to attach electrons at a diffusion-controlled rate; however, the technique used by Baxendale *et al* required a high dose/pulse which contributed to electron-ion recombination. We noted previously that the k_e of CCl_4 measured by Baxendale *et al* is 50-100 percent greater than other published values (40), and we

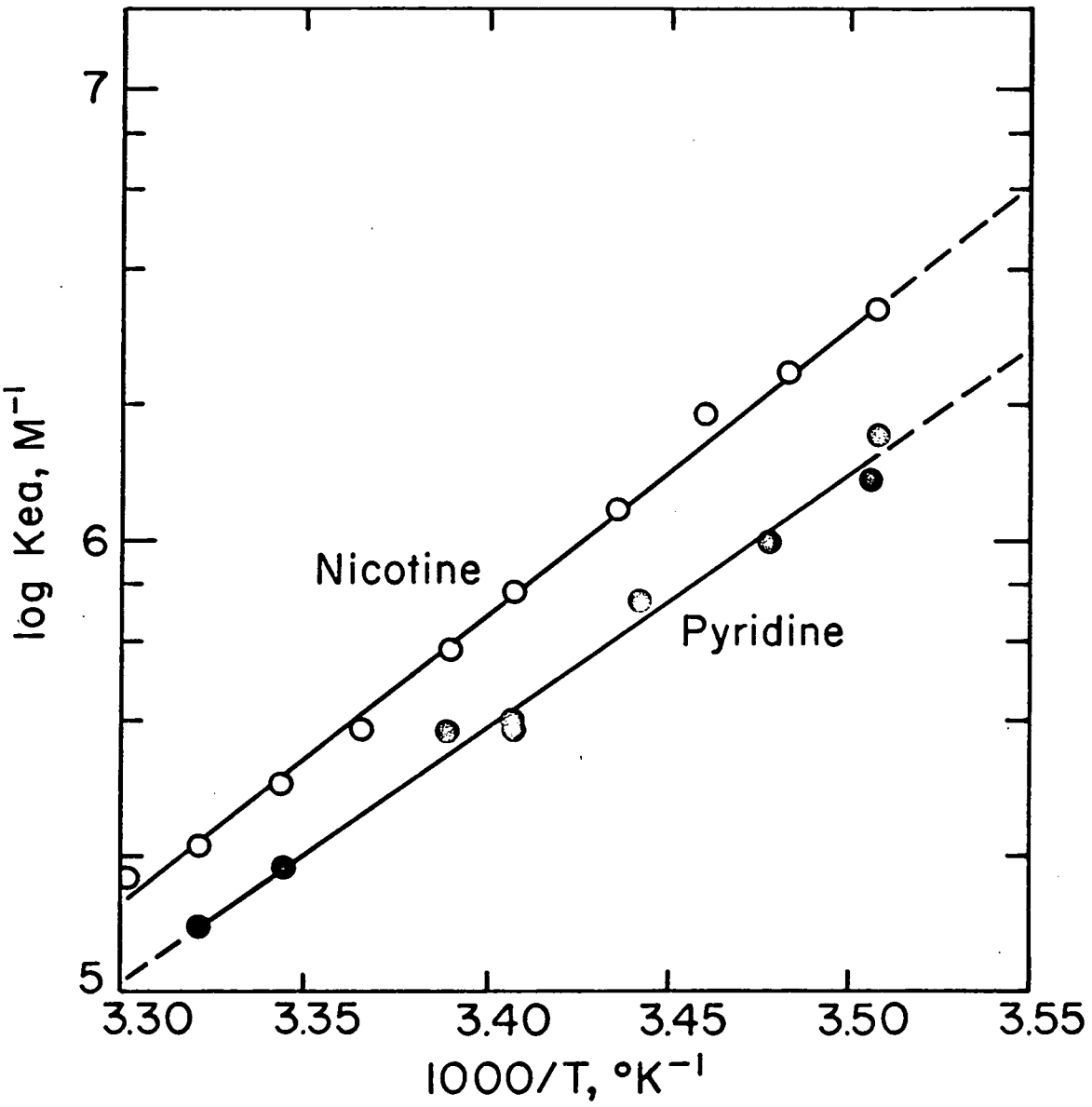

Figure 1. Arrhenius plot of the equilibrium constant, $K = k_1/k_2$, of electron attachment to and detachment from nicotine (O) and pyridine (●) in cyclohexane.

attribute this to uncorrected electron loss by recombination. An electron-ion recombination component to the electron decay curve in their CO_2 measurements could similarly have masked the electron attachment-detachment equilibrium. Our proposed study of CO_2 is also described in detail in the 1981-82 Research Proposal.

D. Picosecond Electron Attachment to Reversed Micelles

The intense interest in aggregates of amphipathic molecules, or micelles, in solution is best exemplified by the 2800 publications on this subject during the last decade (54). This interest primarily stems from the membrane-mimetic characteristics of micelles which provide unique microenvironments that catalyze a variety of chemical reactions (55). Of these micelle-catalyzed reactions, attention has recently focussed on photosensitized electron-transfer processes that are intimately related to photosynthesis and, consequently, solar-energy conversion (56-59). We have recently submitted for publication our report of the first direct observations of quasifree electron attachment to micelles; a preprint of this report is appended [Reference (60), pp. A-58 to A-73 in Appendix], and we shall now briefly describe this study and our current work on micellar solutions.

In an earlier Progress Report (12/1/76-11/30/77), we described our measurements of the mobility, u_m, of reversed micelles and the apparent high quasifree attachment rate of this species. We were unable, however, to directly measure values of k_e because a sufficiently high micellar concentration is required to ensure the formation of micelle aggregates, and this concentration requirement decreased the electron half-lives to the sub-nanosecond time regime which we were unable to resolve. We succeeded in measuring micellar k_e's in our more recent studies, however, by exploiting the time resolution of a recently

developed picosecond pulse-conductivity (PPC) technique to monitor electron lifetimes from ∿50 ps to several hundred ps (60,61).

The structure of many microemulsion and reversed-micellar solutions is poorly understood; therefore, we chose the micelle that has been most thoroughly studied, viz., sodium di(2-ethylhexyl) sulfosuccinate or Aerosol OT (AOT). Heptane could not be used as the solvent as it is in many other studies because u_e in heptane is too low to provide an adequate signal-to-noise ratio for the PPC technique. Consequently, isooctane with u_e = 5.3 cm^2/Vs, which is ∿100 times the u_e of 0.046 cm^2/Vs in n-heptane, was used as the solvent.

The growth of the conductivity signal produced by electrons drifting in an electric field of 20 kV/cm following irradiation of isooctane in the conductivity cell by two fine structure pulses of 16 MeV electrons is shown in Fig. 1a, p. A-71. The exponential decay of the electron signal under the same conditions but with electrons attaching to 6.2×10^{-4} M CCl_4 in isooctane is shown in Figure 1b; the electron half-life $t_{1/2}$ of ∿130 psec derived from this trace yields $k(e^- + CCl_4) = 8.4 \times 10^{12}\ M^{-1}s^{-1}$. Electron attachment to AOT/H_2O reversed micelles in isooctane is illustrated in Figs. 1c and 1d which also show the fourfold enhancement of the attachment rate as the ratio (by weight) R ≡ H_2O/AOT is increased from 0.1 to 1.5. This dependence of the quasifree electron attachment rate or reciprocal $t_{1/2}$ on R is illustrated in Fig. 2 (p. A-72) where

the linear dependence of the attachment rate on the AOT concentration is also shown.

In order to evaluate k_e's of the micelles from the measured $t_{1/2}$'s, the number of AOT molecules per micelle, which we denote by N_{AOT}, must be known. Values of N_{AOT} were measured under conditions similar to those in our experiments by Zulauf and Eicke (62), and their N_{AOT} values are listed in Table I (p. A-69) for the range of R's that we studied. The electron attachment rate constants, k_{obs}'s, were calculated from the measured $t_{1/2}$'s and N_{AOT} values, and these are also listed in Table I and are seen to range from 2-120 x $10^{12} M^{-1} s^{-1}$ over a range of R's from 0.1 to 1.5.

Zulauf and Eicke (62) also measured the radii of AOT/H_2O micelles, r_m's, and these are also included in Table I. These values of r_m and an electron radius, r_e, of 2.7 Å were used in the Smoluchowski equation (Eq. I, p. A-64) to evaluate diffusion-controlled electron attachment rate constants, k_d's, of AOT/H_2O reversed micelles. Values of k_d are listed in Table I and are compared with k_{obs} as a function of r_m in Fig. 3 (p. A-73). This comparison clearly shows that the electron attachment rate does not become diffusion-controlled until an r_m of $\sim$70 Å is reached, which indicates that AOT/H_2O aggregates do not provide a sufficiently deep trap for excess electron attachment at every collision until $\sim$30 H_2O molecules are associated with each AOT. This conlcusion is qualitatively consistent with that of Wong et al who found in their NMR

studies of the structure of water pools in AOT that H_2O molecules do not rotate freely in AOT micelles until the number of H_2O molecules/AOT exceeds ~20 (63).

This study of AOT/H_2O reversed micelles demonstrates not only that thermal electrons are captured by water pools but, more importantly, that the electron lifetime before solvation is dependent upon the degree of structuring of the water in the pools. The latter conclusion is of particular significance to our biologically oriented studies discussed in the next Section. Before discussing these biological studies, however, we shall describe another of our studies of micelles that provides another bridge between our investigations of electrons in model liquids and in biological systems.

Recent work of Menger *et al* has demonstrated that the catalytic activity of enzymes is retained in water pools encapsulated in micelles in liquid hydrocarbon solvents (64). This property of micelles combined with the widely accepted mechanism for the initiation of carcinogenesis which involves the enzymatic activation of procarcinogens to their ultimate reactive forms that are highly electrophilic (65) makes the study of electron attachment to encapsulated procarcinogens and enzymes in micelles of the utmost significance to a better understanding of the mechanism of carcinogenesis.

As a first step in such a study, we measured the quasifree electron $t_{1/2}$ and ion mobility, u_i, in AOT/H_2O reversed micelles in cyclohexane in the presence and absence of

S-9 microsomes. These microsomes, which are extracted from rat liver homogenate (66), contain cytochrome P-450 enzymes that have been shown to be the activating agents that convert procarcinogens to ultimate carcinogens (67) in the Salmonella/mammalian microsome or Ames test, which is currently the best bacterial test available for screening potential carcinogens (68). We found u_i to be 5.1×10^{-5} cm^2/Vs for AOT micelles independent of the concentration of added H_2O or S-9. An electron $t_{1/2}$ of 125 nsec was observed in micellar solutions that contained 0.05 percent AOT and an AOT/H_2O/S-9 ratio (by weight) of 5:1:1; the same $t_{1/2}$ was observed when S-9 was not present. No experiments with procarcinogens added to the micelles have yet been attempted, but such studies are contemplated and are discussed in the 1981-82 Research Proposal.

These preliminary micelle experiments demonstrate the feasibility of studying the effect of structuring of water on the quasifree electron lifetime and of studying the enzymatic conversion of procarcinogens to their ultimate electrophilic derivatives. With this foundation of our quasifree electron transport and reaction studies in model systems completed, we proceed to describe our more biologically oriented research program.

IV. Electrons in Biological Systems

A. Introduction

A considerable fraction of our reseach effort during this triennial reporting period has been directed toward extrapolating the quasifree electron transport and reaction properties of model nonpolar liquids to electronic processes that occur in biological systems. To our knowledge, our laboratory and that of A. Hummel at the Interuniversity Reactor Institute in Delft, The Netherlands, are the only laboratories in which the biological implications of quasifree electrons are being actively investigated.

The key hypothesis involved in these research programs is that water is sufficiently structured in biological systems so that it temporarily appears to quasifree electrons and positive holes to resemble a nonpolar medium. Since these electrons and holes are the most reactive reducing and oxidizing species known, respectively, considerable chemistry and biology can occur before the charge-carriers are solvated in the cellular milieu at which time they are converted to relatively unreactive ionic species.

The Delft group's approach to testing this hypothesis has been to use low temperatures to slow the rotational times of the aqueous solvent and thereby extend the quasifree lifetimes of the charge-carriers. Hummel's group has used this technique to measure the yields and mobilities of quasifree electrons and

holes in frozen aqueous solutions (69,70) and are also attempting to measure the charge transport properties of dry biomolecules (71). Rather than using frozen matrices to extend the quasifree lifetime of charge-carriers, we study the reaction properties of the charge-carriers with solutes of biological interest in a nonsolvating medium. We shall now describe these studies and begin with our radiosensitizer work which involves the possible role of quasifree charges in the sensitization process. This is followed by a summary of our measurements of the diffusion-controlled k_e's of carcinogens, and we conclude with our studies of radiation and chemical induced damage to DNA.

B. Role of Unsolvated Charge-Carriers in Direct Radiosensitization

In our studies prior to this reporting period, we found that radiosensitizers attached quasifree electrons at diffusion-controlled rates (72), whereas all of the biomolecular cellular components that we studied were unreactive toward quasifree electrons (73). This result is now not surprising since the radiosensitization efficiency of sensitizers was subsequently found to correlate with the sensitizers' one-electron reduction potentials (74), and biomolecules are generally considered to be electron-rich and are therefore classified as nucleophiles (65). We used as a base this marked difference in the electron-accepting properties of

radiosensitizers and biomolecules to develop a model of direct radiosensitization in which we demonstrated that unsolvated, or dry, charge-carriers can play an important role in the sensitization process. This model is fully described in the Appendix, pp. A-2 and A-12 to A-16 and is summarized in the following.

As we stated in the Introduction to this Section, a key assumption in our model involves a high degree of solvent structuring in the sheath of water that surrounds the polar biomolecule that is the target of the radiation damage. The extent of structuring and the physical properties of this microenvironment have been a subject of intense debate for more than fifty years (75) and continues to receive considerable attention today (76). A consensus more closely approaching unanimity has been reached on the target of radiation damage, which is now almost universally agreed to be DNA (77).

We assumed DNA to be the target molecule of radiation-induced damage in our direct radiosensitization model and proposed the following main points: (1) dry or unsolvated electrons and positive holes have finite lifetimes during which these charged species have transport and attachment properties similar to analogous charge-carriers in non-polar liquids; (2) the lifetimes of these dry charge-carriers is orders of magnitude longer in the vicinity of polar biomolecules than in bulk water because of a higher degree of structuring of the H_2O

molecules near the biomolecules due to dipole-dipole and ion-dipole interactions; (3) electrons and holes produced by an ionizing event in this structured sheath of water can induce damage to the polar biomolecule that is potentially lethal to the cell before solvation of the charges occurs.

Using this foundation, we demonstrated that several fundamental radiobiological results could be explained. These include the electron affinic nature of radiation sensitizers, the special radiosensitizing properties of oxygen, and the disproportionate fraction of direct radiation damage that is experimentally observed compared to that which is expected from target theory (77).

C. Electron Attachment to Chemical Carcinogens

During the course of our radiosensitizer studies, evidence began to accumulate that many radiosensitizers had mutagenic (78) and carcinogenic (79) properties. These findings were not surprising but rather appeared to be a corollary of Haddow's paradox which states that many cancer chemotherapeutic agents are carcinogenic (80). Since we had found that many radiosensitizers attached electrons at diffusion-controlled rates, we investigated the attachment rates of several classes of carcinogens to determine if k_e's were correlated with carcinogenicity. A full report of our study of carcinogens, which was recently accepted for publication in Cancer Biochemistry Biophysics, is appended; pp. A-38 to A-57. We shall now discuss these results and our associated studies of carcinogens.

We measured the k_e's of 76 chemicals that had been tested for bacterial mutagenicity in at least one of five major studies that were designed to determine the correlation between bacterial mutagenicity and animal carcinogenicity. We found that 37 of 42 carcinogens attached quasifree electrons in cyclohexane at diffusion-controlled rates, whereas the k_e's of 30 of 34 noncarcinogens were significantly less than the diffusion-controlled rate. Our values of the k_e's are listed in Table I, pp. A-51 to A-56, which also includes the bacterial mutagenicity and animal carcinogenicity of the solutes tested.

The mutagenic responses were determined with the Salmonella/microsome or Ames test (66) which, as we stated earlier, p. 34, appears to be the best short-term carcinogen screening test that is currently available (68). The sensitivity (number of positive responses/number of carcinogens tested) and specificity (number of negative responses/number of noncarcinogens tested) of k_e as an indicator of carcinogenicity for the classes of chemicals tested were both 88 percent; see Table II, p. A-58. The sensitivity and specificity may be greater than indicated since several of the "false" positive and "false" negative responses of the k_e test were for chemicals that may have been erroneously classified by animal testing as noncarcinogens and carcinogens, resectively, which we discuss in detail on pp. A-44 to A-46. Nevertheless, the sensitivity and specificity of 88 percent that we found compare favorably with the Ames test results (68) and suggest that the need cited by Bridges of an "empirical 'litmus paper' test, with no known theoretical basis, which (yields) an 80 to 90 percent predictiveness for carcinogenicity" (81) is fulfilled by k_e as an indicator of carcinogenicity.

The acute need of accurate, short-term carcinogen-screening tests to which Bridges refers arises from a combination of several factors. First, epidemiological evidence indicates that >80 percent of all human cancer is traceable to environmental sources (82). This combined with the 20-year latency period for most forms of human cancer appears to be

responsible for the recently discovered (and controversial) 10-percent increase in the national cancer rate (83), which when corrected for the effects of smoking on lung cancer and of sunlight or skin cancer indicates a 25-percent increase in occupationally related cancer (84). Moreover, the post-war chemical industry has been growing at an exponential rate with a doubling time of 8 years which increases the burden of chemicals to which we are exposed (85). The prohibitive cost of $500,000/chemical and the 3-year time requirement of animal tests (86) preclude the luxury of our relying upon this method to identify carcinogens. Thus, the need for an efficacious short-term screening test for carcinogens is evident.

We have already mentioned that the Ames test is considered overall to be the best short-term test that is currently available; however, it should be recognized as also having some serious disadvantages. For example, Ashby and Styles have pointed out that over a three-year period, several chemicals that had yielded "reproducibly negative" responses had changed to "reproducibly positive" (87). These changes in the test responses were due to modifications of one or more of 14 variables in the Ames test system. A study of the carcinogenicity and mutagenicity of 25 polynuclear hydrocarbons by Lijinsky's group in which the Ames-screening system was used to determine the mutagenicity of the chemicals tested indicated that only 58% of the carcinogens were mutagenic and only 41% of

the noncarcinogens were not mutagenic (88). Lijinsky et al suggested that further modifications and improvements of the Ames test would be required for successful screening of carcinogens in this class of chemicals. More recently, Rinkus and Legator analyzed the reported Ames-test mutagenicity and animal-test carcinogenicity results of 465 chemicals and concluded that the Ames test was a poor screen for the carcinogenicity of the following classes of chemicals: azonaphthols; carbamyls and thiocarbamyls; phenyls; benzodioxoles; polychlorinated aromatics; cyclics and aliphatics; steroids; antimetabolites; and symmetrical hydrazines (89). A discussion of problems associated with the Ames test and short-term mutagenicity tests in general has also been presented by de Serres (90).

The main advantage of the Ames test compared to our k_e measurements as a screen for carcinogens is the theoretical basis of the former. A mutagenic response in the Ames assay indicates that the test chemical induced a mutation in the bacterial DNA through either a base-pair or a frameshift mutation (66). The success of the Ames test in correlating bacterial mutagenicity with animal carcinogenicity has rekindled interest in the long dormant somatic mutation theory of cancer which states that the initiating step in the carcinogenesis mechanism is damage to DNA (65,81). The somatic mutation theory is now widely (65) though not universally accepted (91).

Our k_e measurements combined with our model of radiosensitization by dry charge-carrriers leads to a model of carcinogenesis initiation that also involves damage to DNA and is therefore consistent with the somatic mutation theory of cancer. We view the initial DNA-carcinogen interaction as a long range (∿15 Å) quasifree electron transfer reaction from electron-rich DNA to the electrophilic carcinogen. Prior to this step, the carcinogen diffuses in the cellular milieu until it encounters the sheath of water encasing DNA. Diffusion of the carcinogen toward the DNA is then impeded, but electron transport through this quasi-lattice water structure is relatively facile. An electron is transferred from the DNA to the carcinogen and this step initiates bonding between the carcinogen-DNA complex. The adduct thus formed may be an intercalation product or a base-pair or frameshift mutation in the DNA. This model is consistent with recent x-ray diffraction studies of DNA-drug complex hydrates (92) and with studies of charge transport through structured water (93).

A weakness of our model is that it does not discriminate between the efficacy with which procarcinogens and ultimate carcinogens induce DNA damage. Ames incorporated mammalian microsomes that activate procarcinogens to their ultimate reactive forms into his test protocol (66), and this is one of the key elements responsible for the success of the Ames test. Moreover, the accuracy of the Ames test and its requiring

microsomal activation has added further credence to the somatic mutation theory. Olive's recent work that established a correlation between the redox potential and the metabolic reduction rate of nitro compounds (94) is reminiscent of the redox potential-sensitizer efficency correlation (74) described earlier (pp. 36-37) and suggests that quasifree electrons may also play a role in the metabolic activation mechanism. We anticipate that our studies of procarcinogen activation in reversed micelles which are described in Section III.D. and in the 1981-82 Proposal will clarify if under certain conditions ultimate carcinogens attach quasifree electrons more efficiently than procarcinogens and if quasifree electrons are involved in the microsomal activation process. Positive results in either of these studies would make our k_e test for screening carcinogens theoretically more appealing.

Despite the tenuous theoretical basis for the k_e carcinogen test at present, the k_e test has several advantages over the Ames test. Foremost among these is that the k_e measurement is much faster and requires only minutes rather than days to provide an indication of a chemical's carcinogenic properties. Also, the k_e measurement involves a direct measurement of the electron conductivity which makes the test adaptable to electronic read-out devices and to continuous-monitoring applications. The versatility of the test in determining the relative biohazardous potential of complex

mixtures that contain several carcinogens is demonstrated by the following study.

The complex mixtures that we studied were samples of cyclohexane through which the smoke of burning cigarettes had been bubbled. The test protocol involved using a slight vacuum over 100 ml of pure cyclohexane to draw the smoke of a burning cigarette through the solvent. A 50 µl aliquot of the smoke-hexane solution was then added to 50 ml of purified cyclohexane and the solution was degassed in the ion-chamber by 10 minutes of argon-bubbling. The $t_{1/2}$ of the non-volatile, electron-attaching solutes was then measured. A blank with the same protocol except using an unburning instead of a burning cigarette yielded negligible electron attachment. Absolute k_e's could not be calculated since the concentrations of the electron-attaching solutes were unknown; therefore, we estimated the concentration of benzo(a)pyrene that would have been required to produce the observed $t_{1/2}$. The equivalent benzo(a)pyrene concentration measured in the smoke of different types of cigarettes are listed in Table III.

Table III. Carcinogen levels expressed as equivalent concentrations of benzo(a)pyrene, B(a)P, measured in smoke of various types of cigarettes using the quasifree electron attachment technique.

Cigarette	Type	Equivalent [B(a)P] (mg/cigarette)
Golden Lights	low tar, filter	2.1
Merit	low tar, filter	2.3
Marlboro	standard, filter	3.2
Salem	menthol, filter	6.2
Camel	standard, non-filter	7.6

This crude cigarette-smoke test illustrates several important points: (1) the sensitivity of the test - less than .05 percent of the smoke solution from a single cigarette diluted 1,000 times provided a detectable measure of the electron-attaching constituents that were present in the smoke; (2) the versatility of the test - a single $t_{1/2}$ determination provided an apparently meaningful measurement of the concentration of potential biohazards in a complex mixture of carcinogens; and (3) the speed of the test - the burning time of

the cigarette and the degassing time of the test solution were the limiting factors in the k_e measurements. This example should illustrate the value of the electron-attachment test to monitor potentially biohazardous pollution sources such as the smoke-stack effluent from coal-fired electricity-generating plants.

Our final study of carcinogens did not involve k_e measurements but rather utilized the Ames test to determine if mutational synergism occurs when bacteria are exposed simultaneously to ionizing radiation and chemical carcinogens. A study of this nature appears to us to more closely approximate the "real-life" situation of multiple exposures to a host of insults rather than the normal laboratory situation in which a single, well-defined dose of the carcinogen under study is administered. Further, the ubiquitous nature of and the intense interest in low-level radiation makes this study particularly relevant to the public's concern of environmental exposure to carcinogens.

Although the mutagenicity of thousands of chemicals has been tested with the Ames assay, we only know of two reports of the mutagenic response of the Salmonella tester strains to ionizing radiation, and these are in conflict. In one of these, Ames reported a positive mutagenic response for Salmonella exposed to UV, fast neutrons and X-rays (95); in the other, Heddle and Bruce reported a negative mutagenic response of gamma-irradiated Salmonella (96). Details of radiation energy, dose and dose rate were not presented in either study.

The first step in using the Ames test to determine the mutagenic effects of chemical carcinogens and radiation was to establish the radiation dose response of the bacteria which required irradiating the bacteria on plates that contained the

required growth nutrients. Strain TA1537 was plated on rich (Luria) and minimal (E plus glucose salts) plates with added histidine and biotin and the plates were irradiated with Co-60 gamma-radiation at a dose rate of 567 rads/min to doses ranging from 300-1400 rads. The plates were then incubated for 12 hours at 37°C and the survivors counted. The survival-dose curve is presented in Figure 2 where a significant shoulder is seen. No differences were observed between the two types of plates used.

The same strain, TA1537, was then subjected to the normal Ames plate-incorporation protocol (66) with the carcinogen 9-aminoacridine added. This chemical was chosen for our preliminary study because it is the prescribed positive control for TA1537 and does not require metabolic activation (66). Aminoacridine concentrations included 1, 5 and 10 ug/plate and no aminoacridine added for the control.

A more critical set of controls designed to determine the effects of exposure to both radiation and aminoacridine on the bacterial survivor rate was also run. Minimal plates with biotin and histidine added were again used and aminoacridine was added at the same concentrations as in the mutagenicity experiment. Both sets of plates were irradiated with Co-60 at a dose rate of 567 rads/min to doses of 300, 600 and 1000 rads. A set of both types of plates at the same aminoacridine concentrations was also prepared and irradiated at the "low-level" dose rate of 16 rads/hr to a dose of 600 rads. The results are presented in

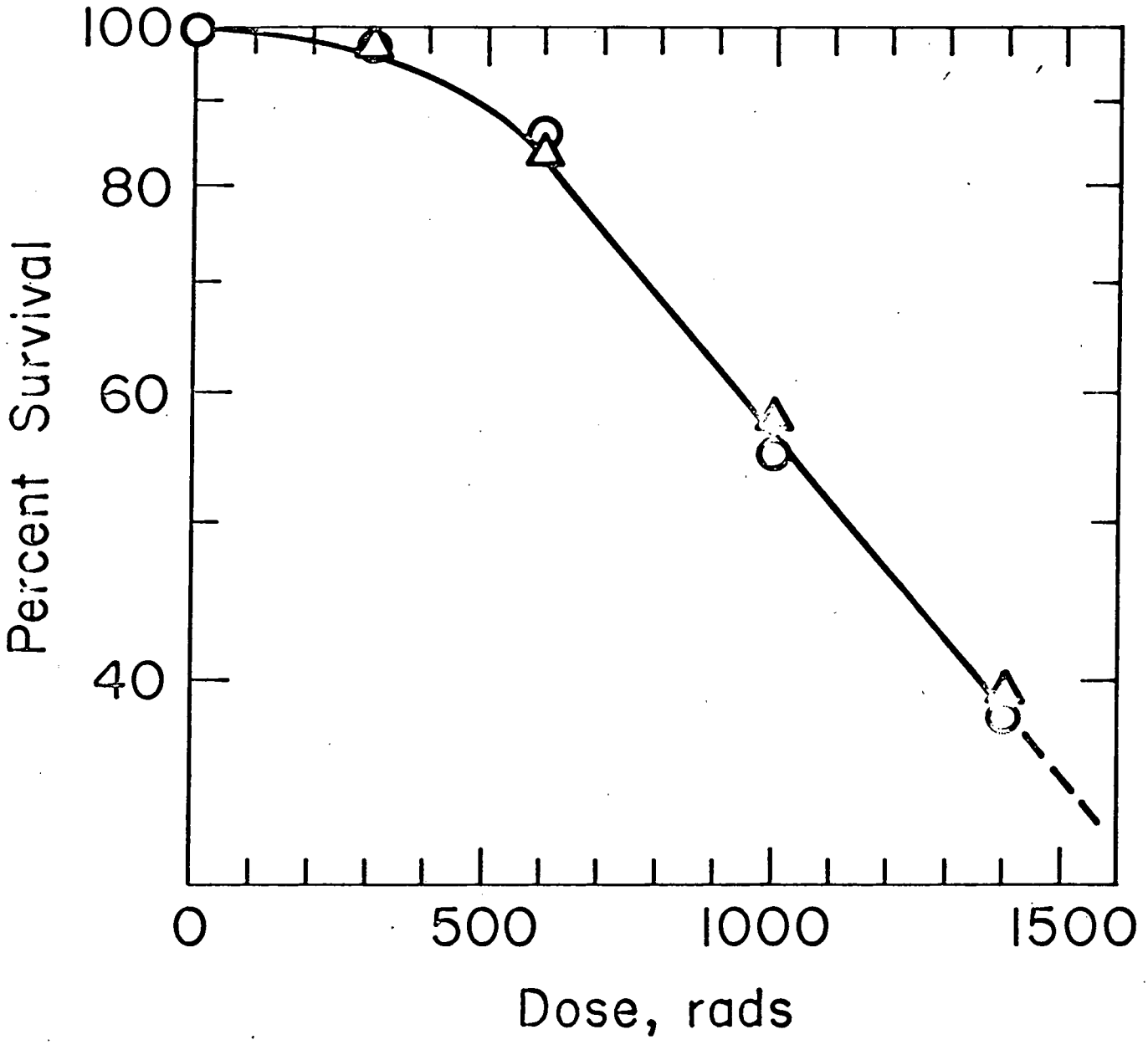

Figure 2. Survival of S. typhimurium TA1537 on rich (Luria), △ and minimal (E plus glucose salts), ○ exposed to Co-60 gamma radiation at a dose rate of 567 rads/min.

Figure 3 where the number of mutants per survivor are plotted *versus* dose.

Before we discuss these results it is first necessary to consider the toxicity of the simultaneous radiation and chemical insults to the bacteria, which is particularly cirtical in this type of assay where not only mutants but also survivors must be measured. In the standard Ames plate-incorporation assay, the appearance of a background lawn of bacterial growth on the plate is the only measure of toxicity that is required (66). If the test chemical is highly toxic, the lawn will be sparse compared to the control background lawn and the test should be repeated at lower chemical concentrations. Even with this lawn-check precaution, however, we have found and Rosenkranz and Poirier have reported (97) that a false-negative response is sometimes observed when mutant deaths are numerous. Consequently, we prefer to follow the recommendation of Kaden *et al* who stress "the absolute necessity of simultaneously measuring the toxicity incurred as a result of treatment" (98).

The problem then arises of simulating the conditions of the minimal growth media as nearly as possible but yet providing sufficient nutrients for mutants to grow. We attempted to accomplish this by measuring bacterial survival on minimal plates with added histidine and biotin, whereas the mutagenicity studies were done according to standard protocol with only traces of histidine present to allow a few colony doublings to

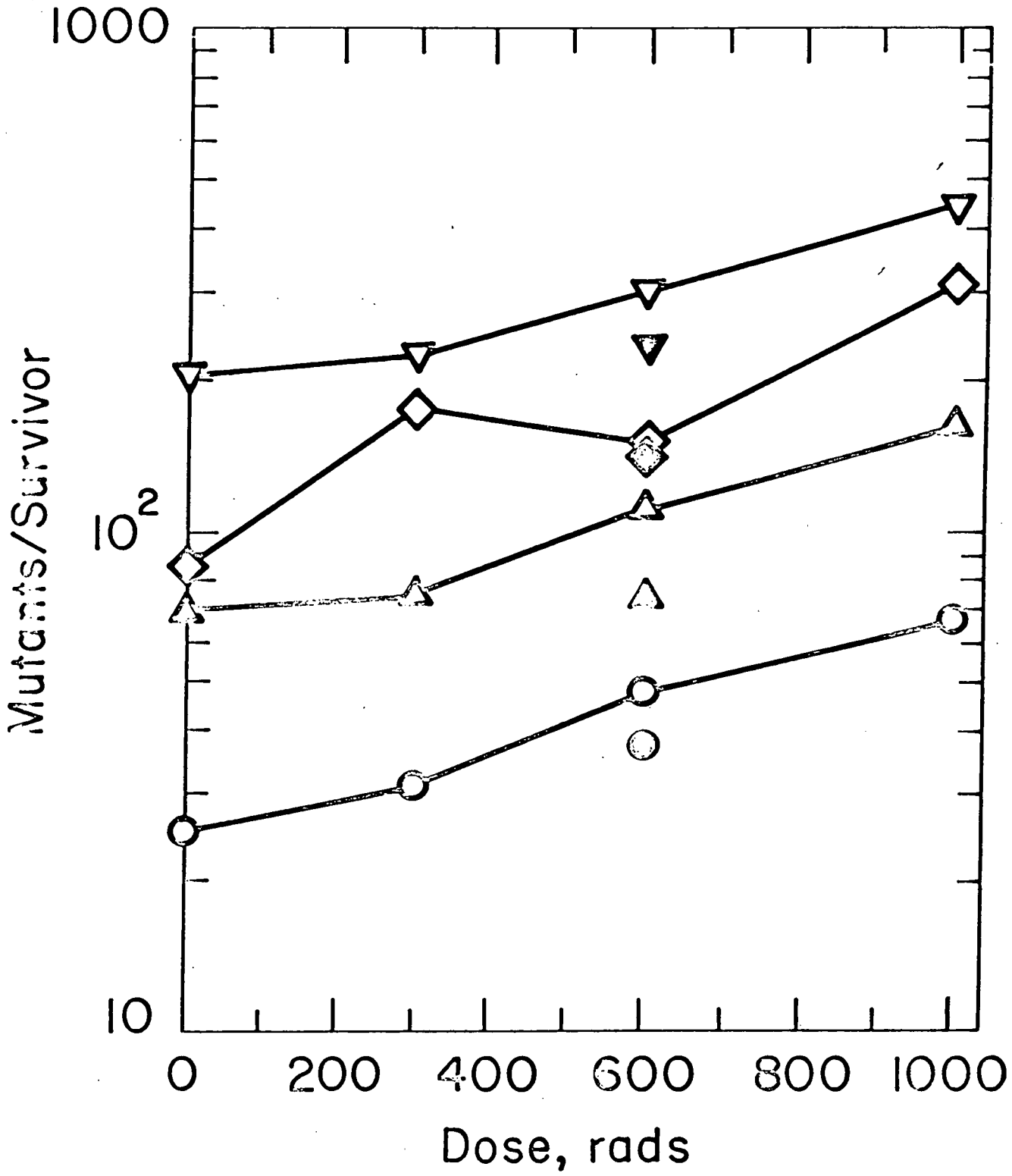

Figure 3. Mutants/survivor of S. typhimurium TA1537 exposed to 9-aminoacridine at zero (○), 1 (△), 5 (◇) and 10 (▽) µg/plate and exposed to Co-60 gamma radiation at 567 rads/min (open symbols) and to X-rays at 16 rads/hr (solid symbols).

form the lawn.

We now return to discuss the results illustrated in Figure 2 and first note that a slight dose-rate effect was observed. The relative mutations/survivor, or m/s were 1.3 ± 0.2 times greater at the two-thousandfold higher dose-rate, which should not be regarded as a significant difference for a single experiment. However it does appear that the repair mechanisms are not markedly dose-rate dependent, and this is a significant conclusion that will be studied more thoroughly in future experiments. An LD_{50} of 1,100 rads is derived from Fig. 1.

We next direct attention to the radiation-only dose-response curve in Fig. 3 which increased nearly exponentially from 25 m/s at zero dose to 67 m/s at 1,000 rads and which yields a doubling dose of 700 rads. This is a different response thanthat obtained by Heddle and Bruce who reported a negative mutagenic response of S. typhimurium to gamma-radiation (96) but appears to be consistent with Ames' finding a positive mutagenic response for several types of ionizing radiation (95). Few details were given in either of these studies, and we infer that no corrections were made for radiation-induced mutant death.

Finally, we consider the simultaneous exposures of TA1537 to 9-aminoacridine (or 9-AA) and radiation. As stated earlier,for radiation only, 25 and 67 m/s were observed at 0 and 1,000 rads, respectively. With 10 ug of 9-AA, 205 and 445 m/s

were observed at the same respective radiation doses. If the 9-AA and radiation mutagenic responses were additive, 67 + 205 = 272 m/s should have been observed with exposures to 10 ug of 9-AA at 1,000 rads, whereas 445 m/s were observed. The enhancement ratio, ER, for the simultaneous exposures is, therefore, 445/272 = 1.6. Values of ER were calculated at other radiation and 9-AA doses and are presented in the following table.

	9-AA, ug		
Dose, rads	1	5	10
300	0.73	1.5	0.94
600	0.95	1.1	1.2
1,000	1.2	2.0	1.6

As is obvious from these results, no spectacular synergism was observed for the mutagenicity of TA1537 exposed to Co-60 gamma radiation and 9-aminoacridine. However, if we exclude the 1 ug 9-AA data, which appear to be in error due to a spuriously large number of mutations observed with no radiation, several significant ER's were observed. These data suggest that the mutagenicity induced in TA1537 by combined exposures to radiation and 9-AA was greater than additive; an effect which warrants further study.

Studies of this nature are extremely time-consuming and

expensive; therefore, we submitted an application to NIH entitled "Radiation Chemical Synergism in Bacterial Mutagenicity" to supplement suppport for this study. The experience that we have gained in conducting the Ames assay will be valuable in the studies described in our 1981-82 DOE Research Proposal in which we propose to use the Ames test and our k measurements to determine the role of quasifree electrons in bacterial mutagenesis.

D. Radiation-Induced Damage in Concentrated DNA Solutions

Our final study related to quasifree electrons in biological systems involves obtaining biochemical evidence that dry charge-carriers play a role in the induction of radiation damage to DNA. As we have already described in Section IV.A. and B, water surrounding DNA has much different physical properties than bulk water and, consequently, the charge-transport properties of these two types of water differ greatly. In most *in vitro* radiobiological studies in which DNA damage is measured, DNA concentrations are orders of magnitude less than the cellular DNA concentration (99); therefore, the observed radiation-induced DNA damage in dilute DNA solutions bears little resemblance to analogous damage in cellular DNA. Since more water-structuring occurs in the concentrated DNA solutions, we initiated studies of radiation-induced damage in concentrated DNA solutions to optimize the experimental conditions for dry

charge-carrier damage and to more closely simulate the cellular environment.

We have conducted experiments in which 4 mM solutions of calf-thymus DNA are irradiated by Co-60 to a dose of 10 krads. This relatively low dose for this type of experiment is used to limit DNA damage to < 1 percent so that the integrity of the DNA-H_2O structure is not lost. Irradiated samples are then analyzed for DNA damage using a technique developed by Ward (100) in which bases released from damaged DNA are measured using a UV (265 nm) monitor. Before the irradiated samples are analyzed for base content, the water solvent is rotary evaporated, the residue is dissolved in tris buffer, the DNA is precipitated with ethanol, and the bulk of the DNA is removed by centrifugation. The supernatant containing the product bases is then rotary evaporated and the residue is dissolved in tris buffer which is then eluted over a Sephadex-A-25 anion exchange column using tris buffer as the eluent. The UV absorbance of the bases are quantitatively measured when eluted from the chromatograph column.

Initial measurements of radiation-induced base release have been made under a variety of conditions, but these experiments were interrupted before reproducible results were obtained. These experiments were conducted by Dr. M. Isildar who returned in March, 1980 to Turkey to fulfill his faculty commitments at the Nuclear Research and Training Institute of

EGE University in Izmir. With these commitments soon to be completed, Dr. Isildar plans to rejoin our group in March, 1981 and will then continue these and other studies designed to elucidate the role of electrons in biological processes. Improvements in the analytical instrumentation used in the base-release measurements will facilitate future measurements and increase the reproducibility of the results.

V. References

1. Shockley, W., "Hot Electrons in Germanium and Ohm's Law", Bell System Tech. J. 30, 990 (1951).
2. Schmidt, W.F. and Bakale, G., "Mobility of Excess Electrons in Liquid Methane", Chem. Phys. Lett. 17, 617 (1972).
3. Bakale, G. and Schmidt, W.F., "Excess Electrons and Positive Charge-Carriers in Liquid Methane. I.", Z. Naturforsch. Teil a28, 511 (1973).
4. Bakale, G. and Schmidt, W.F., "On Excess Electron Transport in Liquid Hydrocarbons", Chem. Phys. Lett. 22, 164 (1973).
5. Sowada, U., Bakale, G., Yoshino, K. and Schmidt, W.F., "Electron Transport in High Mobility Liquid Hydrocarbons and Tetramethylsilane", Proceedings of the 5th International Conference on Conduction and Breakdown in Dielectric Liquids. Noordwijkerhout, The Netherlands, July, 1975.
6. Sowada, U., Bakale, G. and Schmidt, W.F., "Dependence of Electron Mobility on Electric Field Strength", High Energy Chem. 10, 290 (1977).
7. Schmidt, W.F., Bakale, G. and Tauchert, W., "Excess Electrons in Liquid Methane Solutions. II.", Proceedings of the 42nd Conference on Electrical Insulation and Dielectric Phenomena, Montreal, Canada; October, 1973.
8. Bakale, G., Tauchert, W. and Schmidt, W.F., "Electron Transport in Mixtures of Liquid Methane and Ethane", J. Chem. Phys. 63, 4470 (1975).
9. Bagley, B.G., "The Field-Dependent Mobility of Localized Electronic Carriers", Solid State Comm. 8, 345 (1970).
10. Reik, H.G., "Optical Properties of Small Polarons at High Electric Fields", ibid. 8, 1737 (1970).
11. Efros, A.L., "Theory of Kinetic Phenomena in Semiconductors Having a Small Mobility", Sov. Phys.-Sol. State 9, 901 (1967).
12. Bakale, G., Sowada, U. and Schmidt, W.F., "Electron Transport in Low Mobility Liquids Hydrocarbons", 1974 Annual Report, Conference on Electrical Insulation and Dielectric Phenomena, 41, (National Academy of Sciences, Washington, D.C., 1975).
13. Schmidt, W.F., Bakale, G. and Sowada, U., "Excess Electrons and Positive Charge-Carriers in Liquid Ethane", J. Chem. Phys. 61, 5775 (1974).
14. Funabashi, K. and Rao, B.N., "Field-Dependent Mobility in Liquid Hydrocarbons", J. Chem. Phys. 64, 1561 (1976).
15. Tachiya, M., "Electron Transport over Barriers of Fluctuating Height", J. Chem. Phys. 68, 2362 (1978).
16. Döldissen, W., Schmidt, W.F. and Bakale, G., "Excess Electron Mobility in Ethane, Density, Temperature and Electric Field Effects", J. Phys. Chem. 84, 1879 (1980).
17. Döldissen, W., Bakale, G. and Schmidt, W.F., "Excess Electron Mobility in Liquid Ethane at Room Temperature", Chem.

Phys. Lett. 56, 347 (1978).
18. Döldissen, W., Bakale, G. and Schmidt, W.F., "Excess Electron Mobility in Liquid Ethane as a Function of Temperature Between −50 and +26°C", J.Electrostat. 7, 247 (1979).
19. Kimura, T. and Fueki, K., "Effect of Density on Excess Electron Localization in Ethane and Propane", J. Chem. Phys. 66, 366 (1977).
20. Lekner, J., "Mobility Maxima in the Rare-Gas Liquids", Phys. Lett. 27A, 341 (1968).
21. Lekner, J. and Bishop, A.R., "Electron Mobility in Simple Fluids near the Critical Point", Phil. Mag. 27, 297 (1973).
22. Sowada, U., Bakale, G., Yoshino, Y. and Schmidt, W.F., "Electric Field Effect on Electron Capture by SF_6 in Liquid Argon and Xenon", Chem. Phys. Lett. 34, 466 (1975).
23. Bakale, G., Sowada, U. and Schmidt, W.F., "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).
24. Bakale, G., Gregg, E.C. and McCreary, R.D., "Decay of Quasifree Electrons in Pulse-Irradiated Hydrocarbons", J. Chem. Phys. 57, 4246 (1972).
25. Bakale, G., Sowada, U. and Schmidt, W.F., "Electron Attachment to Sulfur Hexafluoride in Nonpolar Liquids", J. Phys. Chem. 79, 3041 (1975).
26. Shibamura, E., Takahashi, T., Kubota, S. and Doke, T., "Ratio of Diffusion Coefficient to Mobility for Electrons in Liquid Argon", Phys. Rev. A, 20, 2547 (1979).
27. Baird,J.K., "Kinetics of Electron Capture by SF_6 in Solution", Can. J. Chem. 55, 2133 (1977).
28. Watson, H.E., Rao, G.G. and Ramaswamy, K.L., "The Dielectric Coefficients of Gases. Part II. The Lower Hydrides of Carbon and Silicon, Oxygen, Nitrogen, Oxides of Nitrogen and Carbon, and Fluorides of Silicon and Sulfur", Proc. Roy. Soc. London 143, 558 (1933-34).
29. Rao, B.N., Bush, R.L. and Funabashi, K., "Field-Dependent Electron Mobility in Methane-Ethane Liquid Mixtures", Can. J. Chem. 55, 1952 (1977).
30. Yakovlev, B.S., Goriev, I.A. and Balakin, A.A., "Temperature Dependence of Electron Scavenging in Liquid Nonpolar Hydrocarbons", Int. J. Radiat. Phys. Chem. 6, 23 (1974).
31. Mozumder, A., "Probability of Escaping Neutralization When the Mobility is Field Dependent'", J. Chem. Phys. 65, 3798 (1976).
32. Baird, J.K., Anderson, V.E. and Rice, S.A., "Comment on 'Probability of Escaping Neutralization When the Mobility is Field Dependent'", J. Chem. Phys. 67, 3842 (1977).
33. Mozumder, A., "'Response to "Comment on 'Probability of Escaping Neutralization When the Mobility is Field Dependent'"'", J. Chem. Phys. 67, 4783 (1977).
34. Mozumder, A. and Carmichael, I., "Effect of Field-Dependent Mobility on the Escape Probability. I. Electrons Photo-

injected in Neopentane", J. Chem. Phys. 68, 3808 (1978).
35. Onsager, L., "Initial Recomination of Ions", Phys. Rev. 54, 554 (1938).
36. Hummel, A., "Ionization in Nonpolar Molecular Liquids by High Energy Electrons", in Advances in Radiation Chemistry, Burton, M. and Magee, J.L., eds., 4, pp. 82-96, (Wiley-Interscience, New York, 1974).
37. Schmidt, W.F. and Pugh, D.R., "On the Polarity Effect of the Breakdown Voltage for Dielectric Liquids in an Inhomogeneous Field", J. Phys. D., Appl. Phys. 10, 1139 (1977).
38. Devins, J.C., Rzad, S.J. and Schwabe, R.J., "The Role of Electronic Processes in the Electrical Breakdown of Insulating Liquids", Can. J. Chem. 55, 1899 (1977).
39. Wong, P. and Forster, E.O., "High-Speed Schlieren Studies of Electrical Breakdown in Liquid Hydrocarbons", ibid., 55, 1890 (1977).
40. Bakale, G., Gregg, E.C., and McCreary, R.D., "Electron Attachment to Nitro Compounds in Liquid Cyclohexane", J. Chem. Phys. 67, 5788 (1977).
41. Allen, A.O., Gangwer, T.E. and Holroyd, R.A., "Chemical Reaction Rates of Quasifree Electrons in Nonpolar Liquids. II", J. Phys. Chem. 79, 25 (1975).
42. Jortner, J. and Gaathon, A., "Effects of Phase Density on Ionization Processes and Electron Localization in Fluids", Can. J. Chem. 55, 1801 (1977).
43. Schindewolf, U., "Physical and Chemical Properties of Dissolved Electrons (Excess Electrons)", Angew. Chem. Intl. Ed. Engl. 17, 887 (1978).
44. Yakovlev, B.S., "Excess Electrons in Non-Polar Molecular Liquids", Russ. Chem. Rev. 48, 615 (1979).
45. Bakale, G., Sowada, U. and Schmidt, W.F., "Negative Ion-Molecule Reactions in Liquid Argon Following Electron Capture by N_2O", Can. J. Chem. 55, 2220 (1977).
46. Holroyd, R.A., Gangwer, T.E. and Allen, A.O., "Chemical Reaction Rates of Quasifree Electrons in Nonpolar Liquids. The Equilibrium $CO_2 + e \rightleftharpoons CO_2^{-*}$", Chem. Phys. Lett. 31, 520 (1975).
47. Warman, J.M., DeHaas, M.P., Zador, E. and Hummel, A., "Concerning the Equilibrium $e^- + \text{Biphenyl} \rightleftharpoons \text{Biphenyl}^-$ in Liquid Tetramethylsilane", ibid., 35, 383 (1975).
48. Holroyd, R.A., "Equilibrium Reactions of Excess Electrons with Aromatics in Nonpolar Solvents", Ber. Bunsenges. Phys. Chem. 81, 298 (1977).
49. Holroyd, R.A., McCreary, R.D. and Bakale, G., "Reversible Reaction of Excess Electrons with p-Difluorobenzene in n-Hexane and Cyclohexane", J. Phys. Chem. 83, 435 (1979).
50. Burrow, P.D., private communication.
51. Frazier, J.R., Christophorou, L.G., Carter, J. G. and Schweinler, H.C., "Negative Ion Resonances in the Fluorobenzenes and Biphenyl", J. Chem. Phys. 69, 3807 (1978).

52. Lin, S., Ph.D. Thesis, University of Houston, 1973.
53. Baxendale, J.H., Keene, J.P. and Rasburn, E.J., "Conductimetric Study of the Kintics of Electrons in Pulse-Irradiated n-Hexane and Cyclohexane at Room Temperature", J. Chem. Soc., Farad. Trans. I, 70, 718 (1974).
54. Menger, F.M., "On the Structure of Micelles", Acc. Chem. Res. 12, 111 (1979).
55. Fendler, J.H., "Surfactant Vesicles as Membrane-Mimetic Agents: Characterization and Utilization", ibid. 13, 7 (1980).
56. Ford, W.A., Otvos, J.W. and Calvin, M., "Photosensitized Electron Transport Across Lipid Vesicle Walls: Quantum Yield Dependence on Sensitizer Concentration", Proc. Natl. Acad. Sci. (USA) 76, 3590 (1979).
57. Kiwi, J. and Grätzel, M., "Chlorophyll A Sensitized Redox Process in Microemulsion Systems", J. Phys. Chem. 84, 1503 (1980).
58. Fendler, J.H., "Microemulsion, Micelles, and Vesicles as Media for Membrane-Mimetic Photochemistry", ibid., 84, 1485 (1980).
59. Gregoritch, S.J. and Thomas, J.K., "Photochemistry in Microemulsions. Photophysical Studies in Oleate/Hexanol/Hexadecane, Oil in Water Microemulsion", ibid., 84, 1491 (1980).
60. Bakale, G., Beck, G. and Thomas, J.K., "Electron Capture in Water Pools of Reversed Micelles", submitted to J. Phys. Chem., October 17, 1980 and included in Appendix, pp. A-58 to A-73.
61. Beck, G., "Fast Conductivity Techniques in Pulse Radiolysis", Proc. 6th Int. Cong. Radiat. Res., Okada, S., Imamura, M., Terashima, T. and Yamaguchi, H., eds., 279-286 (Toppan, Ltd., Tokyo, 1979).
62. Zulauf, M. and Eicke, H.-F., "Inverted Micelles and Microemulsions in the Ternary System H_2O/Aersol-OT/Isooctane as Studied by Photon Correlation Spectroscopy", J. Phys. Chem. 83, 480 (1979).
63. Wong, M., Thomas, J.K. and Nowak, T., "Structure and State of H_2O in Reversed Micelles. 3", J. Am. Chem. Soc. 99, 4730 (1977).
64. Menger, F.M. and Yamada, K., "Enzyme Catalysis in Water Pools", ibid., 101, 6731 (1979).
65. (a) Miller, J.A. and Miller, E.C., "Persepctives on the Metabolism of Chemical Carcinogens", in Environmental Carcinogenesis, Emmelot, P. and Kriek, E., eds. pp. 25-50 (Elsevier, Amsterdam, 1979);
(b) Miller, E.C., "Some Current Perspectives on Chemical Carcinogenesis in Humans and Experimental Animals: Presidential Address", Cancer Res. 38, 1479 (1978).
66. Ames, B.N., McCann, J. and Yamasaki, E., "Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian Microsome Mutagenicity Test", Mutat. Res. 31, 347

(1975).
67. For a recent review of metabolic activation of procarcinogens in short-term screening tests, see Wright A.S., "The Role of Metabolism in Chemical Mutagenesis and Chemical Carcinogenesis", Mutat. Res. 75, 215 (1980).
68. Poirier, L.A. and de Serres, F.J., "Initial National Cancer Institute Studies on Mutagenesis as a Prescreen for Chemical Carcinogens: An Appraisal", J. Natl. Cancer Inst. 62, 919 (1979).
69. Verberne, J.B., Loman, H., Warman, J.M., deHaas, M.P., Hummel, A. and Prinsen, L., "Excess Electrons in Ice", Nature 272, 343 (1978).
70. Warman, J., deHaas, M.P. and Verberne, J.B., "Decay Kinetics of Excess Elecrons in Crystalline Ice", J. Phys. Chem. 84, 1240 (1980).
71. Hummel, A., Warman, J.M. and deHaas, M.P., private communication.
72. Bakale, G., Gregg, E.C., and McCreary, R.D., "Structural Effects on the Electron Attachment Rates of Nitroaromatic Molecules and Implications to Radiation Sensitization", Radiation Research Society-Health Physics Society Joint Meeting, San Francisco, CA, June 27-July 2, 1976.
73. Gregg, E.C., McCreary, R.D. and Bakale, G., "Effect of Biologically Important Solutes on Charge-Carrier Decay Processes in n-Hexane", presented at the Fifth International Congress of Radiation Research, Seattle, WA, July 14-24, 1974.
74. Adams, G.E., Flockhart, I.R., Smithen, C.E., Stratford, I.J., Wardman, P. and Watts, M.E., "Electron-Affinic Sensitization. VII. A Correlation between Structures, One-Electron Reduction Potentials, and Efficiencies of Nitroimidazoles as Hypoxic Cell Radiosensitizers", Radiat. Res. 67, 9 (1976).
75. For a review of the history of the structured-water controversy, see Ling, G.N., "Hydration of Macromolecules", Water and Aqueous Solutions, Horne, R.A., ed., 663-700 (Wiley-Interscience, N.Y., 1972).
76. For recent comprehensive reviews of water-biomolecule interactions, see: Pethig, R., Dielectric and Electronic Properties of Biological Materials (J. Wiley & Sons, N.Y., 1979) or Drost-Hansen, W. and Clegg, J., eds., Cell-Associated Water (Academic Press, N.Y., 1979).
77. Adams, G.E., "Radiation Chemical Mechanisms in Radiation Biology", Advances in Radiation Chemistry 3, Burton, M. and Magee, J.L., eds., 125-208 (J. Wiley, N.Y., 1972).
78. Chin, J.B., Sheinin, D.M.K. and Rauth, A.M., "Screening for Mutagenicity of Nitro-Group Containing Hypoxic Cell Radiosensitizers Using Salmonella typhimurium Strains TA100 and TA98", Mutat. Res. 58, 1 (1978).

79. Rustia, M. and Shubick, P., "Introduction of Lung Tumors and Malignant Lymphomas in Mice by Metronidazole", J. Natl. Cancer Inst. 48, 721 (1972).
80. Haddow, A., Harris, R.J.C., Kon, G.A.R. and Roe, E.M.F., "The Growth-Inhibitory and Carcinogenic Properties of 4-Aminostilbene and Derivatives", Phil. Trans. Roy. Soc. London A, 1241 (1948).
81. Bridges, B.A., "Short-Term Screening Tests for Carcinogens", Nature 261, 195 (1976).
82. Wynder, E.L. and Gori, G.B., "Contribution of the Environment to Cancer Incidence: An Epidemiological Exercise", J. Natl. Cancer Inst. 58, 825 (1977).
83. Pollack, E.S. and Horm, J.W., "Trends in Cancer Incidence and Mortality in the United States, 1969-1976", ibid. 64, 1091-1103 (1980).
84. Schneiderman, M.A., cited in Smith, R.J., Science 209, 998 (1980).
85. Davis, D.L. and Magee, G.N., "Cancer and Industrial Chemical Production", ibid. 206, 1356 (1979).
86. Rosenkranz, H.S., seminar at Case Western Reserve University, October 9, 1980.
87. Ashby, J. and Styles, J.A., "Factors Influencing Mutagenic Potency In Vitro", Nature 274, 20 (1978).
88. Andrew, A.W., Thibault, L.H. and Lijinksy, W., "The Relationship Between Carcinogenicity and Mutagenicity of Some Polynuclear Hydrocarbons", Mutat. Res. 51, 311 (1978).
89. Rinkus, S.J. and Legator, M.S., "Chemical Characterization of 465 Known or Suspected Carcinogens and Their Correlation with Mutagenic Activity in the Salmonella Typhimurium System", Cancer Res. 39, 3289 (1979).
90. de Serres, F.J., "Problems Associated with the Application of Short-Term Tests for Mutagenicity in Mass-Screening Programs", Environ. Mutagen. 1, 203 (1979).
91. Rubin, H., "Is Somatic Mutation the Major Mechanism of Malignant Transformation", J. Natl. Cancer Inst. 64, 994 (1980).
92. Neidle, S., Berman, H.M. and Shieh, H.S., "Highly Structured Water Network in Crystals of a Deoxydinucleoside-Drug Complex", Nature 288, 129 (1980).
93. Edmonds, D.T., "A Reversible Electrostatic Channel for Ion Transport", Chem. Phys. Lett. 65, 429 (1979).
94. Olive, P.N., "Correlation between Metabolic Reduction Rates and Electron Affinity of Nitroheterocycles", Cancer Res. 39, 4512 (1979).
95. Ames, B.N., "A Bacterial System for Detecting Mutagens and Carcinogens", in Mutagenic Effects of Environmental Contaminants, Sutton, E. and Harris, M., eds., pp. 57-66 (Academic, New York, 1972).
96. Heddle, J.A. and Bruce, W.R., "Comparison of Tests for Mutagenicity or Carcinogenicity Using Assays for Sperm Abnor-

malities, Formation of Micronuclei, and Mutation in Salmonella", in Origins of Human Cancer, Hiatt, H., Watson, J.D. and Winstein, J.A., eds., pp. 1549-1557 (Cold Spring Harbor Laboratory, Cold Spring, N.Y., 1977).

97. Rosenkranz, H.S. and Poirier, L.A., "Evaluation of the Mutagenicity and DNA-Modifying Activity of Carcinogens and Noncarcinogens in Microbial Systems", J. Natl. Cancer Inst. 62, 873 (1979).

98. Kaden, D.A., Hites, R.A. and Thilly, W.G., "Mutagenicity of Soot and Associated Polycyclic Aromatic Hydrocarbons to Salmonella typhimurium", Cancer Res. 39, 4152 (1979).

99. Lehninger, A.L., Biochemistry. The Molecular Basis of Cell Structure and Function, pp. 29 and 636 (Worth Publishers, Inc., New York, 1970).

100. Ward, J.F. and Kuo, I., "Strand Breaks, Base Release and Post-Irradiation Changes in DNA γ-irradiated in Dilute O_2-Saturated Aqueous Solutions", Radiat. Res. 66, 485 (1976).

APPENDIX

Presentations, papers published and submitted, and activities pertinent to the "Ionization in Liquids" project from September 1, 1977 to April 30, 1981.

A. Abstracts of Presentations

A-1. . . . .Bakale, G. and Gregg, E.C., "Conjecture on the Role of Dry Electrons in Direct Radiosensitization", presented at the Eighth L.H. Gray Memorial Conference, Cambridge, England, September 5-9, 1977.

A-2. . . . .Bakale, G. and Gregg, E.C., "Dry Electrons and Holes in Radiobiological Damage", presented at the Biophysical Society and American Physical Society Joint Meeting, Washington, D.C., March 27-30, 1978.

A-3. . . . .Döldissen, W., Schmidt, W.F. and Bakale, G., "Excess Electron Mobility in Liquid Ethane from -36°C to the Critical Temperature", presented at the 6th International Conference on Conductivity and Breakdown in Dielectric Liquids, Rouen, France, July 24-28, 1978.

A-4. . . . .Bakale, G., McCreary, R.D. and Gregg, E.C.,

"Quasifree Electron Attachment to Carcinogens in Liquids", presented at the 28th Annual Meeting of the Radiation Research Society, New Orleans, LA, June 1-5, 1980.

Preprints + Reprints removed.

B. Papers Published

A-5. . . . .Bakale, G., Gregg, E.C. and McCreary, R.D., "Electron Attachment to Nitro Compounds in Liquid Cyclohexane", J. Chem. Phys. 67, 5788 (1977).

A-12 . . . .Bakale, G. and Gregg, E.C., "Conjecture on the Role of Dry Charges in Radiosensitization", Br. J. Cancer, Suppl. III, 37, 24 (1978).

A-17 . . . .Döldissen, W., Bakale, G. and Schmidt, W.F., "Excess Electron Mobility in Liquid Ethane at Room Temperature", Chem. Phys. Lett. 56, 347 (1978).

A-19 . . . .Döldissen, W., Bakale, G. and Schmidt, W.F., "Excess Electron Mobility in Liquid Ethane as a Function of Temeprature Between -50 and +26°C," J. Electrostat. 7, 247 (1979).

A-25 . . . .Holroyd, R.A., McCreary, R.D., and Bakale, G., "Reversible Reaction of Excess Electrons with p-Difluorobenzene in n-Hexane and Cyclohexane", J. Phys. Chem. 83, 435 (1979).

A-30 . . . .Döldissen, W., Schmidt, W.F. and Bakale, G., "Excess Electron Mobility in Ethane, Density, Temeprature and Electric Field Effects", J. Phys. Chem. 84, 1879 (1980).

C. Manuscripts Submitted for Publication

A-38 . . . .Bakale, G., McCreary, R.D. and Gregg, E.C., "Quasifree Electron Attachment to Carcinogens in Liquid Cyclohexane", in press, Cancer Biochem. Biophys. 5 (2), 000 (1981).

A-58 . . . .Bakale, G., Beck, G. and Thomas, J.K., "Electron Capture in Water Pools of Reversed Micelles", in press, to J. Phys. Chem., 1981.

A-74 . . . .Bakale, G. and Schmidt, W.F., "Effect of an Electric Field on Electron Attachment to SF_6 in Liquid Ethane and Propane", to be submitted

D. Other Activities

1. Seminars presented

(a) Bakale, G., "Role of Bioelectronics in Radiobiology

and Carcinogenesis", presented at Case Western Reserve University, Division of Radiation Biology, March 21, 1978.

(b) Bakale, G., "Electrons and Holes in Radiation Biology and Carcinogenesis", presented at the Interuniversity Reactor Institute, Delft, The Netherlands, August 28, 1978.

(c) Isildar, M., "γ-Radiolysis of DNA in Oxygenated Aqueous Solutions: Alterations at the Sugar Backbone and Base Liberation", presented at Case Western Reserve University, Division of Radiation Biology, January 25, 1980 and the University of Notre Dame, Notre Dame, IN, February 5, 1980.

(d) Bakale, G., "Fast Electron Reactions in Liquids", Solar Energy Research Institute, Golden, CO, March 20, 1980.

(e) Bakale, G., "Quasifree Electron Attachment to Carcinogens", presented at Case Western Reserve University, Division of Radiation Biology, July 18, 1980.

2. Guest appointments of G. Bakale at the Hahn-Meitner Institute für Kernforschung, Bereich Strahlenchemie, West Berlin, Germany.

(a) June 1 - October 31, 1977.

(b) May 15 - August 31, 1978.

(c) November 1, 1979 - January 14, 1980.

(d) march 15 - May 15, 1981 (proposed).

3. Miscellaneous

(a) Bakale, G., American Chemical Society Short Course on "Chemical Carcinogenesis", Chicago, November 16-17, 1978.

(b) Bakale, G., NIH General Research Support Review Committee of the State Uniersity of New York, College of Old Westbury, Westbury, NY, September 7-8, 1979.

(c) Bakale, G., "Discussion of Electron Transport and Attachment in Liquids and Biological Systems", The Interuniversity Reactor Institute, Delft, The Netherlands, October 29-31, 1979.

(d) Bakale, G., XIII Radiological and Chemical Physics Contractors Meeting, Richland, WA, Marh 17-18, 1980.