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DHEW CA 19283: "Core Support for Radiation Oncology Research"
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SUMMARY

We have made significant progress in better understanding the electron transport and reaction properties of model liquids and biological systems since the May 1, 1981 initiation date of our current DOE contract. These new results complement our earlier studies of electrons and are important to all aspects of the interaction of radiation with matter and to the myriad of electronic processes that occur in a variety of systems. These processes range from dielectric breakdown to carcinogenesis as is illustrated in the following list of electron transport and reaction studies that we have conducted in this contract period. Our studies included:

1. Effect of an external electric field on the electron attachment rate constant, k_e , of SF_6 observed in the molecular liquids ethane and propane.
2. Implications of field-dependent k_e 's to dielectric breakdown in liquids.
3. Influence of the electron-acceptor dipole moment on k_e in *i*-octane and the effect of solvent on electron-dipole interaction.
4. Solvent effects on the electron-attachment process through measurements of the k_e of CCl_4 and ethyl bromide in *i*-octane/TMS mixtures.
5. Electron attachment to reversed-micellar aggregates and comparison of the influence of the micellar water pool on the

electron-capture process in the solvents i-octane and TMS.

6. Correlation of diffusion-controlled k_e 's with carcinogenicity for use as a carcinogen screening test and the effect of solvents on the k_e -carcinogenicity correlation.
7. Determination of the bacterial mutagenicity of a series of nitroaromatic compounds which have diffusion-controlled k_e 's.
8. Determination of radiation-induced mutagenesis in the Ames' Salmonella tester strains TA1535, TA98 and TA100.

The preceeding list illustrates the interdisciplinary nature of our proposed research program but the interdependence of the eight studies listed can only be realized by a complete reading of the following Progress Report.

PROGRESS REPORT

I. Introduction

The study of processes that occur subsequent to the interaction of ionizing radiation with matter has received increasing attention since the discovery of X-rays by Roentgen in 1895. Knowledge of these post-ionization processes is essential to apply most efficaciously the effects of radiation that range from benevolent applications such as the diagnostic and therapeutic effects of radiation to destructive applications such as the neutron bomb which is intended to maximize injury to living systems. For either of these extremes or for numerous other applications, knowledge of the physics and chemistry of radiolytic reactants is required to attain the maximum desired effect of ionizing radiation.

Progress in understanding the reactions of reactive intermediates produced by radiation has been limited by the techniques available to monitor these reactions. Initially, only gross radiation effects such as the fogging of photographic film or the analysis of stable radiolytic products was available, but the development of pulse radiolytic techniques now permits us to monitor the reactions of radiolytic species to within a few picoseconds of their production in the ionizing event.

Of the various species produced by radiation, electrons are the most reactive and are involved in the widest variety of reactions. Early in our Ionization in Liquids project, we developed a pulse-conductivity technique to monitor the decay rate of electrons and to measure the rate constant of electron attachment, k_e (1). We also obtained a great deal of information of the electron transport properties of liquids through measurements of the electron mobility, u_e , and have studied the dependence of u_e on the electric field, E , to elucidate details of the electron transport mechanism (2). We shall now report our progress within the last year of studying electron reactions in model liquids and in biologically relevant systems. Further, we shall

report our initial results of bacterial mutagenicity studies that are aimed at determining the role of electrons in the induction of damage to DNA.

II. Electron Attachment in Model Liquids

A. Field-Dependent Electron Attachment

Our earlier studies of the dependence of u_e on E in molecular liquids provided novel information on electron transport (2) that was subsequently used in theoretical studies to clarify the electron-transport mechanism (3). In addition, our studies of field-dependent k_e 's in the atomic liquids argon and xenon (4) complemented earlier electron-transport studies in these liquids (5). Analogously, we undertook a study of the field dependence of k_e in the molecular liquids ethane and propane which was designed to complement our earlier field-dependent u_e studies. We have recently concluded this study and a full report of this work and its implications to dielectric breakdown are appended, pp. A-1 to A-5 and A-6 to A-8, respectively. We shall now briefly summarize this work.

For our field-dependent study of k_e in molecular liquids, we chose ethane and propane as the solvents since the high-field u_e behavior in these liquids had been thoroughly studied (2 c-h), and we chose SF_6 as the solute since the electron-accepting properties of this molecule had been shown to be diffusion-controlled over the range of u_e 's that were studied (6). The pulse-conductivity technique described earlier (1,6) was again used to measure k_e 's.

Several examples of the enhancement of k_e by E are illustrated in Fig. 1, p.A-2. This enhancement of k_e was found to occur at $E > E_c$, where E_c is the critical field at which u_e had been found to be field-dependent (2c-h). The proportional increase of k_e with D_e , which is expected for diffusion-controlled electron attachment under the experimental conditions used, is illustrated in Figures 2 (p.A-2) and 3 (p.A-3) for ethane and propane, respectively. The slopes of these k_e

vs. D_e plots yields an electron- SF_6 encounter radius, R , of 14.5 Å.

This empirically derived R is significantly greater than the hard-core radius of SF_6 , which is 2.9 Å, and is also greater than the induced-dipole electron-capture radius, r_α , of SF_6 which we found to be 7 Å (see p.A-4). The difference between R and r_α may be due to a breakdown of the electron-induced dipole interaction equations at distances of 10-20 Å as suggested by Baird (7); however, we suggest an alternative explanation for $R \approx 2 r_\alpha$ which is based on our earlier electron-transport studies.

From our field-dependent u_e studies in ethane, propane, and ethane-methane mixtures, we derived for λ , the electron-jump distance between solvent localization sites, values ranging from 7-40 Å. Subsequent considerations of our u_e (E) data by Funabasni et al. (3e,g) and Tachiya (3f) indicated that a value of ~ 10 Å was more appropriate. If we accept this value of λ and identify it with the electron-reaction radius R_e at which electrons "instantaneously" jump to the SF_6 capture radius of 7 Å, a range of values of R from 10-17 Å is derived. This distribution of R 's yields a mean R of 13.5 Å which is in good agreement with the empirical value of 14.5 Å.

Perhaps the most significant conclusion to draw from this work is that field-dependent electron attachment is restricted not only to the special case of high-mobility atomic liquids (4), but is also important in electron reactions in molecular liquids. Consequently, it should be generally true that the reactions of electrons produced by an ionizing event in any media are spatially dependent upon the distance of the electron from the parent ion. This spatial dependence of k_e has heretofore been ignored in radiation physics, chemistry and biology.

Our field-dependent k_e studies also have implications to dielectric-breakdown theory, and a full report of this subject is appended, pp. A-6 to A-8. SF_6 has been found to modify the breakdown strength of transformer oils (8a) which may

be due to SF_6 attaching electrons that are liberated in the pre-breakdown stage (8b). Our results indicate that SF_6 should inhibit the initiation of breakdown in transformer oils at fields from 100-250 kV/cm, but at higher fields at which electron are heated by the field prior to attachment, the efficiency of SF_6 to attach electrons decreases. This is due to the attachment cross-section of SF_6 having a sharp maximum at thermal energies (9), and we have observed in liquid argon and xenon that the k_e of SF_6 decreases markedly at high fields where electron heating occurs (4). Consequently, the addition of a hot-electron scavenger to SF_6 -inhibited transformer oil should significantly enhance the breakdown strength of the mixture. Analogous suggestions for increasing the breakdown strengths of gaseous dielectrics using fluorocarbons have been made by Christophorou et al. (10).

B. Electron Attachment to Polar Solutes

Since the first direct measurements of quasifree-electron attachment rate constants less than ten years ago (1,11), numerous studies of electron attachment to a variety of solutes in solvents in which u_e varied more than a thousandfold have provided considerable insight into the electron-attachment mechanism. These studies have revealed that k_e is strongly influenced by the electron conduction-band energy, V_o , of the solvent (12) and, consequently, these attachment studies have contributed to a better understanding of electron transport in disordered media. However, questions concerning the basis for the k_e - V_o interdependence still remain (12c cf. 13), and this approach has provided little information concerning the dynamics of electron-solute interaction. We have attempted to obtain the latter type of information by an alternative approach.

The nitro- functional group, $-\text{NO}_2$, is a powerful electron-withdrawing group that has proven to be valuable to physical-organic chemists in deducing reaction mechanisms and to radiation biologists in designing "electron affinic, oxygen-mimetic" hypoxic-cell radiosensitizers (14). The electron-withdrawing effect of $-\text{NO}_2$ is

illustrated in Table I by the dipole moment, μ , that it induces when substituted on otherwise nonpolar aliphatic molecules, e.g. $\mu(\text{CH}_4) = 0$ cf. $\mu(\text{CH}_3\text{NO}_2) = 3.5\text{D}$. These non-attaching aliphatic solutes are converted to efficient electron scavengers when nitrated as also is shown in Table I in which the k_e 's of several nitroaliphatics are compared with CCl_4 in the solvents n-hexane, c-hexane and isooctane. In our earlier study of electron attachment to mono-substituted nitrobenzenes in cyclohexane, we obtained the first evidence that electron-dipole interaction influences the electron-attachment rate (15), an effect that prior to our study had been considered to be negligible in liquids (13). We have now extended this study of electron-dipole interaction to the solvent isooctane to determine if the dipole moment of the solute also influences k_e in a higher mobility liquid.

The newly measured values of k_e for ortho-, meta- and para-substituted nitrobenzene derivatives in isooctane are compared with our previously measured values of k_e in c-hexane in Table II. As was found for nitrobenzenes in c-hexane, the dipole moment of the solute also strongly influences k_e in i-octane and, in fact, the dipole effect on k_e is generally more pronounced in i-octane. For example, $k_e(\text{ortho})/k_e(\text{para})$ of dinitrobenzene is $6.7/2.8 = 2.4$ in c-hexane and $53/7.3 = 7.3$ in i-octane; similarly the same ratio for fluoronitrobenzene is $5.7/3.4 = 1.7$ in c-hexane and $37/13 = 2.8$ in i-octane. This enhancement of the dipole effect on k_e in the higher mobility liquid [$(u_e(\text{i-C}_8) = 5.2$ cf. $u_e(\text{c-C}_6) = 0.22 \text{ cm}^2/\text{Vs}$ at 21°C (12 b)] does not concur with our earlier prediction that electron-dipole interaction would be diminished in higher mobility liquids (15), and we are currently attempting to determine the cause of this effect. In addition, we are attempting to establish if the three-to eightfold increase of k_e in i-octane compared to c-hexane is consistent with the k_e - V_0 correlations of Schiller (13) or those of Holroyd (12c).

Table I. Dipole moments and rate constants of electron attachment to CCl_4 and nitroaliphatic solutes in n-hexane, c-hexane and i-octane at 20°C.

<u>Solute</u>	<u>μ, D^a</u>	$k_e \times 10^{-12}, \text{M}^{-1} \text{s}^{-1}$		
		<u>n-C_6^b</u>	<u>c-C_6^b</u>	<u>i-C_8^c</u>
CCl_4	0.0	1.2	3.0	6.6
nitromethane	3.5	0.35	1.2	16
1-nitropropane	3.5	1.3	3.5	18
nitrocyclohexane	3.6	1.3	3.3	13

(a) B.F. Levine and C.G. Bethea, J. Chem. Phys. 63, 2666 (1975).

(b) Reference (15).

(c) This work.

Table II. Rates constants of electron attachment measured in c-hexane and 1-octane at 20°C and dipole moments^a of monosubstituted nitrobenzenes.

R-nitrobenzene	-R	Position	μ^a, D	$k_e \times 10^{-12}$	
				c-C ₆	1-C ₈
Electron-withdrawing-R					
nitrobenzene	-	-	4.1	5.1	34
dinitrobenzene	-NO ₂	<u>ortho</u>	6.1	6.7	53
		<u>meta</u>	3.9	5.0	34
		<u>para</u>	0.5	2.8	7.3
nitrobenzonitrile	-CN	o-	6.2	6.6	41
		m-	3.8	5.9	34
		p-	0.7	4.0	34
nitrobenzaldehyde	-CHO	o-	4.3	5.5	27
		m-	3.3	4.8	20
		p-	2.4	3.7	19
nitroacetophenone	-COCH ₃	o-	4.7	5.3	27
		m-	3.7	5.2	13
		p-	3.5	4.8	25
α, α, α -trifluoronitrotoluene	-CF ₃	o-	5.7 ^b	5.9	35
		m-	3.5 ^b	5.2	17
		p-	1.5 ^b	-	22
Halogens					
fluoronitrobenzene	-F	o-	5.0 ^c	5.7	37
		m-	3.6 ^c	5.0	30
		p-	2.6	3.4	13
chloronitrobenzene	-Cl	o-	4.2	5.8	35
		m-	3.4	4.4	21
		p-	2.6	4.5	15

Table II. (Continued)

bromonitrobenzene	-Br	o-	4.2	6.1	47
		m-	3.4	5.3	43
		p-	2.7	5.0	35
iodonitrobenzene	-I	o-	3.8	5.1	40
		m-	3.4	4.6	28
		p-	2.9	4.4	25
Electron-releasing-R					
nitrotoluene	-CH ₃	o-	3.7	5.0	33
		m-	4.2	5.8	35
		p-	4.4	5.3	30
nitroanisole	-OCH ₃	o-	4.9	6.1	31
		m-	3.9	4.9	23
		p-	4.8	5.8	32
nitroaniline	-NH ₂	o-	4.1	4.1	11
		m-	4.9	4.9	-
		p-	5.5	-	14
N,N-dimethylnitroaniline	N(CH ₃) ₂	o-	4.2	5.6	23
		m-	5.3	5.3	13
		p-	6.9	6.7	40

^a Unless noted otherwise, values of μ from A. L. McClellan, "Tables of Experimental Dipole Moments" (Freeman, San Francisco, 1963).

^b Calculated from bond moments of V.I. Minkin, O.A. Osipov, and Y.A. Zhdanov, "Dipole Moments in Organic Chemistry", edited by W.E. Vaughan (Plenum, New York, 1970), pp. 93-94.

^c B.F. Levine and C.G. Bethea, J. Chem. Phys. 63, 2666 (1975).

C. Electron Attachment in i-Octane/TMS Mixtures

Although numerous studies of electron mobility in solvents having different electron transport properties have contributed significantly to electron-transport theory, another important contribution to this area has been made from the few studies of u_e in mixtures of solvents that have dissimilar electron-transport properties; e.g. n-hexane/neopentane (16) and methane/ethane (2g). Consequently, we inferred that an electron-attachment study in mixtures of two-solvents, each of which have well-defined but dissimilar electron-transport properties, would yield new information on both electron-transport and electron-attachment processes in disordered media.

This type of study requires the measurement of k_e of numerous samples at different solvent ratios; therefore, the picosecond pulse-conductivity technique developed by G. Beck (17) was used. We chose tetramethylsilane, TMS, as the high-mobility solvent [$u_e = 100 \text{ cm}^2/\text{Vs}$ at 21°C (12b)] and i-octane as the low-mobility solvent [$u_e = 5.2 \text{ cm}^2/\text{Vs}$ at 21°C (12b)]. Ethyl bromide (EtBr) and CCl_4 were chosen as the solutes; these solutes have similar values of k_e in i- C_8 but the k_e 's differ by a factor of 1000 in TMS (12a,b). We were unable to use a liquid having a lower mobility than isooctane such as c-hexane [$u_e = 0.22 \text{ cm}^2/\text{Vs}$ at 21°C (12b)] because the picosecond pulse-conductivity technique is not sufficiently sensitive to monitor the decay of electrons having a $u_e < 2 \text{ cm}^2/\text{Vs}$. All k_e measurements were done at $21 \pm 2^\circ\text{C}$.

The values of k_e of EtBr and CCl_4 in pure i-octane and TMS that we measured are compared with literature values in Table III. The agreement is good except for the case of EtBr in TMS, and we are currently attempting to discover the source of this discrepancy. The nearly exponential dependence of the k_e of EtBr and CCl_4 with the mole fraction of i-octane is illustrated in Figure 1. We are uncertain at this time if the minimum in the k_e - mole fraction plot at $\sim 90\%$ i-octane is real or anomalous and shall establish this before publication of the results.

Minday et al observed an exponential dependence of u_e on the mole fraction

Table III. Comparison of values of k_e measured in this work^a with published values of k_e for EtBr and CCl₄ in i-octane and TMS at 19-22°C.

<u>Solvent/Solute:</u>	<u>$k_e \times 10^{-12}, M^{-1}s^{-1}$</u>	
	<u>EtBr</u>	<u>CCl₄</u>
i-octane	5.5 ^a	8.7 ^a
	5.1 ^b	8.9 ^c
TMS	0.067 ^a	52.0 ^a
	0.042 ^d	54.0 ^d

a. This work, G. Bakale and G. Beck, to be published, 1982.

b. Reference (12b)

c. Reference (19)

d. Reference (12a)

Figure 1. Dependence of the electron attachment rate constants of CCl_4 (O) and ethyl bromide (Δ) on the mole fraction of i-octane in i-octane/TMS mixtures at 21°C .

of n-hexane in n-hexane/neopentane mixtures (16). If we assume that the same relationship is applicable to i-octane/TMS mixtures, we can interpolate values of u_e for the mixture and combine these with our measured values of k_e to determine the dependence of k_e on u_e . The result is illustrated in Figure 2, which in addition to our k_e -mixture data includes published values of k_e that extend the plot to the u_e of n-hexane.

Figure 2 vividly illustrates the marked influence that the medium has on the electron-attachment properties of solutes. Whereas in low-mobility solvents the k_e 's of EtBr and CCl_4 are identical within experimental error, the electron-attachment rates of these solutes diverge at $u_e > 5 \text{ cm}^2/\text{Vs}$ and reach nearly a thousandfold difference at $u_e = 100 \text{ cm}^2/\text{Vs}$. Another feature of the k_e - u_e dependence which Figure 2 illustrates is the square-root dependence of the k_e of CCl_4 on the electron mobility as is demonstrated by the solid line which is drawn with a slope of 0.5. The $k_e \propto \sqrt{u_e}$ dependence was originally noted by Beck and Thomas nearly ten years ago (11) but no physical significance has yet been attached to this relationship.

A final point to note concerning Figure 2 is the k_e of EtBr and CCl_4 in pure neopentane compared to the k_e 's of the same solutes in the i-octane/TMS mixture in which u_e is equivalent, i.e. $70 \text{ cm}^2/\text{Vs}$ (2c). The k_e of CCl_4 is $2.9 \times 10^{13} \text{ M}^{-1}\text{s}^{-1}$ in pure neopentane (12a) which is significantly less than the k_e of 4.2×10^{13} that we measured in the i- C_8 /TMS mixture. The ~40 percent difference appears to be beyond the experimental error of the two k_e studies, but an even greater difference in the k_e 's of EtBr was observed, viz. 3.4×10^{11} (12a) in neopentane compared to $1.5 \times 10^{11} \text{ M}^{-1}\text{s}^{-1}$ in the i- C_8 /TMS mixture. This twofold difference demonstrates that the attachment process is not influenced to an equal extent by the factors that influence electron transport. To determine if another physical property of the medium would better match the k_e 's in the different solvents, we shall next consider the conduction-band energy of the liquids, V_o .

Figure 2. Dependence of the electron attachment rate constants of CCl_4 (○, ●) and ethyl bromide (△, ▲) on the electron mobility in *i*-octane/TMS mixtures (open symbols) and in the pure liquids indicated (solid symbols) at 21°C; solid line is drawn with a slope of 0.5.

Holroyd and Tauchert found that the V_o of binary mixtures varied linearly with the mole fraction of the components for both n-hexane/TMS and n-hexane/neopentane solutions (18). If we assume that the same linear dependence of V_o applies to the mole fraction in i-octane/TMS mixtures, we can then determine the dependence of k_e on V_o for this two-component system. The k_e - V_o dependence is illustrated in Figure 3 which has been extended to n-hexane using the V_o data of Holroyd et al (12c).

Similarities between Figures 2 and 3 are apparent which is expected if one considers that Figure 2 is a plot of $\log k_e$ vs. $\log u_e$ and a linear dependence has been found between $\log u_e$ and V_o (12c). However, one should note the deviations of the k_e 's of EtBr and CCl_4 at the V_o of pure neopentane compared to the same V_o in the i-octane/TMS mixtures. For EtBr, the k_e in neopentane is again $3.4 \times 10^{11} \text{ M}^{-1} \text{ s}^{-1}$ (12a) which is markedly less than the k_e in the mixture which is $8 \times 10^{11} \text{ M}^{-1} \text{ s}^{-1}$. Also, the k_e of CCl_4 in neopentane differs by about twofold from the k_e in the mixture; the k_e values are 2.9×10^{13} (12a) and $1.5 \times 10^{13} \text{ M}^{-1} \text{ s}^{-1}$ in neopentane and i- C_8 /TMS, respectively. Thus, we conclude that the V_o of the mixture does not sufficiently describe the electron-solvent interactions that influence electron attachment. We are currently considering these results and shall attempt to extract a more quantitative interpretation of our observations.

D. Picosecond Electron Attachment to Reversed Micelles

As described in last year's Progress Report, we have conducted the first study of quasifree-electron attachment to reversed micelles by using the same picosecond pulse-conductivity technique that we employed in the i- C_8 /TMS study described in the preceeding Section. This work was done in collaboration with Dr. G. Beck of the Hahn-Meitner Institute, West Berlin and Dr. J.K. Thomas of the Notre Dame University Chemistry Department. A full report of this initial study of electron attachment to Aerosol-OT (AOT) reversed micelles is appended, pp. A-9 to A-11, and we shall now briefly summarize this work before describing our more recent studies of

Figure 3. Dependence of the electron attachment rate constants of CCl_4 ($\circ, \bullet$) and ethyl bromide ($\Delta, \blacktriangle$) on the conduction-band energy in i-octane/TMS mixtures (open symbols) and in the pure liquids indicated (solid symbols) at 21°C.

reversed micelles in TMS.

AOT was chosen as the solute in our initial study because it is the most thoroughly studied reversed-micelle and i-octane was used as the solvent since the u_e in i-octane is greater than that of other solvents commonly used in reversed-micelle studies. Examples of the conductivity signal growth and decay in the presence of CCl_4 and AOT are shown in Figure 1 of the appended paper, p. A-10. The effect of increasing the ratio of $\text{H}_2\text{O}/\text{AOT}$, which we define as R , is illustrated in Figure 2, p. A-11, at values of R ranging from 0 to 1.5. As R is increased, the amount of H_2O solubilized by the AOT is increased and this leads to a larger micellar radius, r_m . Values of r_m for our experimental conditions are listed in Table I, p. A-11, as are values of N_{AOT} , the number of AOT molecules per micelle; both the r_m and N_{AOT} values were obtained from the measurements of Zulauf and Eicke (20).

We used the Smoluchowski equation to calculate the diffusion-controlled electron-attachment rate constant, k_d . Values of k_d are compared with our measured values of electron attachment in Table I and in Figure 3, p. A-11, which shows that the attachment rate becomes diffusion-controlled when the micellar radius is $>70 \text{ \AA}$. This observation indicates that the $\text{AOT}/\text{H}_2\text{O}$ aggregates do not attach electrons at every collision until $\sim 30 \text{ H}_2\text{O}$ molecules are associated with each molecule of AOT.

In our more recent collaboration with Beck and Thomas we again studied electron attachment to AOT reversed micelles but we used TMS as the solvent. We demonstrated in Section II.C that the electron-attachment process is strongly influenced by the medium, and we studied k_e 's in AOT/TMS solutions to determine if electrons with a u_e of $100 \text{ cm}^2/\text{Vs}$ would react at diffusion-controlled rates with the large electron traps that the $\text{H}_2\text{O}/\text{AOT}$ aggregates provide. If the attachment process were diffusion-controlled in this system, we anticipated that k_e would exceed $10^{15} \text{ M}^{-1}\text{s}^{-1}$ and be one of the fastest reactions ever observed.

The linear dependence of the electron-attachment rate, or reciprocal

electron half-life $t_{1/2}$, on the AOT concentration is shown in Figure 4 in which the influence of H_2O on the attachment rate is also illustrated. We found that the H_2O/AOT micelles in TMS became unstable at $R > 1.1$ and thus we were limited in the amount of H_2O that was added to AOT. The slopes of the $1/t_{1/2}$ vs AOT plots were used to evaluate empirical rate constants, k_o 's, with the equation

$$k_o = \ln 2 / t_{1/2} [AOT] \quad (I)$$

where $[AOT]$ is the concentration of AOT expressed in moles/liter. Values of k_o obtained in this manner are listed in Table IV. In order to convert these empirical attachment rates to electron-attachment rate constants of micelles, which we denote by k_m the aggregation number of the micelles, N_{AOT} , must be known. Values of N_{AOT} for AOT in TMS were independently measured by Dr. Thomas (21) and these are listed in Table IV. Values of k_m calculated with the equation:

$$k_m = N_{AOT} k_o \quad (II)$$

are also listed in Table IV.

The dependence of k_m on R in the solvents *i*-octane and TMS are compared in Figure 5 where one sees that the micellar electron-attachment rate is much greater in *i*-octane than in TMS at low values of R but a reversal in the relative k_m 's occurs at $R > 1$. To some extent this effect reflects the dependence of N_{AOT} on R ; i.e. from a comparison of the N_{AOT} values for TMS listed in Table IV with those for *i*-octane in Table I, p. A-11, one sees that at low values of R , $N_{AOT} (i-C_8) > N_{AOT} (TMS)$ whereas at $R > 1$, $N_{AOT} (TMS) > N_{AOT} (i-C_8)$. The larger micellar aggregate provides a larger attachment target for the electron, or in terms of the Smoluchowski equation, a larger encounter radius. Unfortunately, the AOT radii in TMS have not been measured and,

Figure 4. Dependence of $1/t_{1/2}$ on $[AOT]$ at H_2O/AOT ratios ranging from 0 to 1.1 in TMS at $21^\circ C$.

Table IV. Values of rate constants of electron attachment to AOT molecules, k_o , and to AOT micelles, k_m , having aggregation numbers N_A measured in TMS at 21°C.

R <u>(H₂O/AOT)</u>	$k_o \times 10^{-11}$ <u>(M⁻¹s⁻¹)</u>	N_A^a <u>(AOT/micelle)</u>	$k_m \times 10^{-13}$ <u>(M⁻¹s⁻¹)</u>
0	0.60	4.7	0.10
0.05	0.93	5.6	0.17
0.1	1.7	6.8	0.35
0.2	5.1	9.9	1.3
0.3	7.7	15	2.6
0.5	17	32	9.1
1.1	47	300	90

(a) J.K. Thomas, private communication

Figure 5. Comparison of the dependence of the electron attachment rate constants of H₂O/AOT micelles on R in i-octane (○) and TMS (△) at 21°C.

thus, we cannot compare the observed k_m values with the diffusion-limited rate constants as we did in the AOT/i-octane study. Perhaps our AOT/TMS work will encourage micellar chemists to study the $H_2O/AOT/TMS$ reversed-micelle system and measure the micellar radii.

III. Electrons in Biological Systems

A. Introduction

The interdisciplinary nature of our Ionization in Liquids project is best exemplified by our initiating the use of the Ames Salmonella mutagenicity assay (22) to study the role of electrons in bacterial mutagenesis. The prominent cancer researcher B.A. Bridges has stated that "detecting DNA damage is merely a very sensitive way of detecting electrophilic reagents" (23), and the Ames bioassay is the best established and most sensitive method of detecting damage to DNA in a living system. Therefore, we plan to utilize this assay and our knowledge of electron reactions to replace Bridges' ambiguous term "electrophilic" with physico-chemical terminology that should lead to a more fundamental understanding of the factors that control electron reactions in biological systems.

Another significant addition to our Ionization in Liquids project during the last year has been the return of Dr. Mahmut Isildar to our group. Several recent publications of Dr. Isildar are included in the Appendix, and he has resumed his studies of radiation-induced damage to DNA and, in addition, has collaborated in our Ames mutagenicity studies that are described in Sections III.C-D. Before describing these biological studies, however, we shall first report the progress we have made in our studies of electron attachment to carcinogens in c-hexane and i-octane.

B. Electron Attachment to Chemical Carcinogens

A full report of our electron-attachment study of 42 carcinogens and 34 noncarcinogens in c-hexane has been published and is appended, pp. A-12 to A-18. We found that in c-hexane 37 of 42 carcinogens had diffusion-controlled k_e 's whereas the k_e 's for 30 of 34 noncarcinogens were significantly less than diffusion-controlled. The k_e 's that we measured for the 76 chemicals studied are listed in Table I, pp. A-14

and A-15. Included in this table are the animal-test carcinogenicity and Ames-test mutagenicity responses of all the chemicals studied.

The electron-attachment responses are compared with the Ames-test responses for each chemical class in Table II, p. A-19. This summarization table indicates that the sensitivity (number of positive responses/number of carcinogens tested) and specificity (number of negative responses/number of noncarcinogens tested) for the k_e -screening test is 88 percent, which compares favorably with the Ames-test accuracy. The sensitivity and specificity that we found suggests 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" (23), is fulfilled by our k_e -carcinogen screening test in c-hexane.

In an attempt to improve the sensitivity and specificity of the k_e test, we conducted an analogous study of the k_e 's of well-defined carcinogens and noncarcinogens in another solvent, replacing c-hexane with i-octane. Our model-liquid studies described in Section II demonstrated that the solvent significantly influences electron attachment to solutes, and from Figure 2 one sees that the diffusion-controlled k_e in i-octane is about threefold greater than in c-hexane. We expected that with this greater range for electron attachment to occur before the diffusion-controlled limit was reached, several of the false-positive responses that were obtained in c-hexane would change to correct-negative.

The k_e 's in i-octane of 38 carcinogens and 27 noncarcinogens are listed in Table V which also includes the carcinogenicity, C, as determined in animal tests and bacterial mutagenicity, M, as determined in the Ames Salmonella test. All chemicals were chosen from the five comprehensive mutagenicity studies that we used as our basis of solute selection in our c-hexane study (24-28). The k_e 's range from $<10^8$ to $>10^{13}$ $M^{-1}s^{-1}$; this millionfold range in k_e 's is the same order as Ames et al noted for the range of bacterial mutagenicity response (24). Several of the k_e 's of carcinogens are

about tenfold greater than we had measured in c-hexane.

In the c-hexane study, we used the diffusion-controlled k_e of CCl_4 of $2.9 \times 10^{12} \text{ M}^{-1}\text{s}^{-1}$ as the boundary separating a positive and negative response. In i-octane this border-line appears to be more ambiguous, but for consistency, we again used the k_e of CCl_4 , which in i-octane is $6.6 \times 10^{12} \text{ M}^{-1}\text{s}^{-1}$, as the boundary separating positive and negative responses. Using this criterion of $6.6 \times 10^{12} \text{ M}^{-1}\text{s}^{-1}$ as separating positive and negative responses, the k_e response, R, for 65 solutes is also listed in Table V.

In addition to the response "+" and "0", one also sees listed in Table V two responses labeled "?". These are for the carcinogens 2- and 4-aminobiphenyl which yielded positive R's in c-hexane, but in i-octane we observed an electron attachment-detachment equilibrium with the aminobiphenyls which was analogous to that which we and others observed in other solute/solvent systems (29). We are unclear at this time whether the equilibrium should be classified as a positive or negative response; therefore, we chose for this preliminary report to consider this an indeterminate response denoted by "?".

To obtain the "accuracy" or "predictiveness" of k_e as a screening technique for carcinogens, we again determined the sensitivity, which is the number of positive responses per number of carcinogens tested, and the specificity, which is the number of negative responses observed per number of noncarcinogens tested (30). The sensitivity and specificity by chemical class for the k_e carcinogenicity test in i-octane are presented in Table VI and overall are found to be 83 and 78 percent, respectively, which is significantly less than the 88 percent values that we observed for these criteria in c-hexane (see Table II, p. A-16). However, we may find that a dividing line separating positive and negative responses other than the k_e of CCl_4 may be more appropriate for the i-octane study, and, therefore, we defer concluding which of the solvents is better for screening carcinogens.

Table V. Comparison of k_e and R^1 measured in isooctane at 20°C with carcinogenicity, C^2 , and mutagenicity, M^2 , of chemicals.

A. Aromatic Amines

<u>Chemical</u>	<u>k_e ($M^{-1}s^{-1}$)</u>	<u>R^1</u>	<u>C^2</u>	<u>M^2</u>	<u>References</u> ³
1. aniline	<0.01	0	0	0	a-e
2. p-aminophenol	<0.01	0	0	0	a
3. 3,3',5,5'-tetramethyl-benzidine	0.025	0	0	0	a,c
4. p-anisidine	<0.01	0	0	0	c
5. o-aminobenzoic acid	0.16	0	0	0	b,c
6. dicyclohexylamine	<0.0002	0	0	0	c
7. α -naphthylamine	1.2	+	0	+0 ⁵	a,b,e/c,d ⁵
8. β -naphthylamine	1.1	+	+	+0	a-c,e/d
9. p-aminoazobenzene	0.65	+	+	+0	a-c,e/d
10. o-aminoazotoluene	0.64	+	+	+	a,b
11. 2-aminobiphenyl	eq'm	?	+	+0	a,c,e/d
12. 4-aminobiphenyl	eq'm	?	+	+	a-e

B. Polycyclic Aromatics

1. naphthalene	0.55	0	0	0	a,c
2. anthracene	0.60	0	0	0	a-e
3. fluorene	1.7	+	0	0	a,b
4. pyrene	0.95	+	0	0	a,b
5. chrysene	1.1	+	+	+0	a/d,e
6. benz[e]pyrene	1.5	+	+	+0	a,e/d
7. benz[a]pyrene	1.4	+	+	+	a-e
8. benz[a]anthracene	0.37	0	+	+0	a,c,e/d
9. 9,10-dimethylanthracene	1.1	+	+	+	c

<u>Chemical</u>	<u>k_e ($M^{-1}s^{-1}$)</u>	<u>R^1</u>	<u>C^2</u>	<u>M^2</u>	<u>References</u> ³
C. Nitro-aromatics					
1. 2-nitrobiphenyl	2.6	+	+	+	c
2. 4-nitrobiphenyl	4.0	+	+	+ / 0	a, c, e / d
3. 2-nitronaphthalene	0.93	+	+	+	a, d, e
4. 2-nitrofluorene	3.7	+	+	+	a, c-e
5. 4-nitroquinoline-1-oxide	0.68	+	+	+	a-e
D. Nitrosamines					
1. N-nitrosodimethylamine	2.9	+	+	+ / 0	a, b / c
2. N-nitrosodiethylamine	3.6	+	+	+ / 0	a-c / d, e
3. N-nitrosodipropylamine	3.3	+	+	+	a, b
4. N-nitrosodibutylamine	2.6	+	+	+	a, b
5. N-nitrosodiphenylamine	3.8	+	+	0	b-e
6. N-nitrosopyrrolidine	1.6	+	+	+	a
7. N-nitrosopiperidine	5.3	+	+	+	a
8. N-methyl-N'-nitrosoguanidine	0.54	+	+	+	a-e
9. N-nitroso-N-methylurethane	0.68	+	+	+	a
E. Alkyl & Aromatic Halides					
1. chloroacetic acid	0.12	0	0	0	a
2. carbon tetrachloride	0.66	+	+	0	a
3. dimethylcarbonyl chloride	0.20	0	+	+	a, c-e
4. benzyl chloride	2.8	+	+	+ / 0	a, d / e
5. Dieldrin	4.7	+	+ / 0 ⁴	0	a / c
6. methyl iodide	2.6		+	+ / 0	a, d / e
7. p,p'-DDE	3.6		+	0	a
8. hexachlorocyclohexane	5.1		+	0	c
9. Folpet	2.8	+	0	+	a
10. Captan	6.3		+	+	a

<u>Chemical</u>	<u>k_e ($M^{-1}s^{-1}$)</u>	<u>R¹</u>	<u>C²</u>	<u>M²</u>	<u>References³</u>
F. Esters, Epoxides, Carbamates, Amides					
1. glycidol	<0.005	0	0	+	a,d,e
2. 1,2:7,8-diepoxyoctane	<0.01	0	+	+	a
3. urethane	<0.004	0	+	+/?	c/a,d,e
4. acetamide		0	+	0	a,d,e
5. thioacetamide	1.4	+	+	0	a,d,e
6. acetanilide	<0.01	0	0	0	b
G. Heteroaromatics					
1. indole	<0.003	0	0	0	c
2. carbazole	0.9	+	0	0	c
3. quinoline	0.9	+	+	+	b
H. Alcohols, Diols, Sterols, Acids					
1. ethanol	<10 ⁻⁵	0	0	0	a
2. ethylene glycol	<0.001	0	0	0	a
3. resorcinol	<0.01	0	0	0	a,c
4. diethylstibesterol	0.22	0	+	0	c
5. benzoic acid	0.21	0	0	0	a,c
6. gibberellic acid	<0.1	0	0	0	a
I. Miscellaneous					
1. cyclohexane	<10 ⁻⁵	0	0	0	a
2. toluene	10 ⁻⁴	0	0	0	c
3. acetone	<0.01	0	0	0	a
4. dimethyl sulfoxide	<0.001	0	0	0	a
5. t-stilbene	1.1	+	0	0	b

Table V Legend:

- (1) R denotes response of electron-attachment test with $R = +$ or 0 for k_e greater or less than $6.6 \times 10^{12} \text{ M}^{-1}\text{s}^{-1}$, respectively.
- (2) Positive (+) and negative (0) responses listed in columns C and M are based upon carcinogenicity, C, in animal and/or epidemiological studies and mutagenicity, M, in the Ames test as reported in references indicated.
- (3) References:
  - (a) McCann et al, (24)
  - (b) Sugimura et al, (25).
  - (c) Purchase et al, (26).
  - (d) Rosenkranz and Poirier, (27).
  - (e) Simmon, (28).
- (4) Dieldrin was classified as a carcinogen in (a) and a noncarcinogen in (c); in 1974 the IARC concluded that Dieldrin is an hepatocarcinogen in mice and therefore is classified as a carcinogen in this study.

The sensitivities and specificities of the Ames test as a carcinogen screen are also listed in Table VI and found to be 69 to 92 percent, respectively. Thus, the Ames test yields significantly fewer false-positives than the k_e test; however, this better predictiveness for noncarcinogens is counter-balanced by the greater number of false-negative responses that are observed for carcinogens. One should also note that 13 of 65 or 20 percent of the Ames test results are indeterminate; i.e. both a negative and a positive mutagenic response were reported for one-fifth of the chemicals tested. This ambiguity is considerably greater than the 2 of 65 responses that we found to be indeterminate and is reflective of the number of variables that influences the response of the Ames test (31). For an excellent recent critique of the Ames test and a rebuttal to that critique by Ames, we refer the reader to Reference (32).

C. Mutagenicity of Nitrobenzene Derivatives

Consideration of the excellent correlations between the carcinogenicity and the diffusion-controlled k_e 's of chemicals which we described in the preceding Section together with the diffusion-controlled k_e 's of nitrobenzene derivatives that we measured as described in Section II.B. and Reference (15) led us to conclude that a thorough study of the mutagenic properties of nitroaromatics should be conducted to determine if these compounds have heretofore unrecognized mutagenic, and therefore possibly carcinogenic, properties. Despite the drawbacks of the Ames Salmonella test that we described in the preceding Section, the consensus is that the Ames test is the best overall biological test that is currently available for screening mutagens/carcinogens if one considers the factors of the sensitivity, specificity and expense of the test and the time required for testing (33).

Table VI. Comparison of sensitivity (+/+)^a, specificity (0/0)^b and indeterminate (?)^c results of k_e vs. Ames testing of 65 chemicals.

Chemical Class	k_e			Ames		
	+/+	0/0	?	+/+	0/0	?
A. Aromatic amines	3/3	6/7	2	2/2	6/6	4
B. Polycyclic aromatics	4/5	2/4	0	2/2	4/4	3
C. Nitroaromatics	5/5	0/0	0	4/4	0	1
D. Nitrosamines	9/9	0/0	0	6/7	0/0	2
E. Alkyl & Aromatics Halides	7/8	1/2	0	2/6	1/2	2
F. Esters, Epoxides, Etc.	1/4	2/2	0	1/3	1/2	1
G. Heteroaromatics	1/1	1/2	0	1/1	2/2	0
H. Alcohols, Diols, Etc.	0/1	5/5	0	0/1	5/5	0
I. Miscellaneous	0/0	4/5	0	0/0	5/5	0
TOTAL:	30/36	21/27	2/65	18/26	24/26	13/65
PERCENTAGE:	83	78	3.0	69	92	20

(a) Sensitivity is number of positive responses per number of carcinogens tested; i.e. $R = +/C = +$

(b) Specificity is number of negative responses per number of noncarcinogens tested; i.e. $R = 0/C=0$

(c) See text for discussion of indeterminate results.

In order to ensure that we applied the Ames-test protocol correctly in testing the nitro-compounds, we first chose those nitrobenzene derivatives from among those for which mutagenicity data were available. Of the nitro compounds for which we had measured values of k_e in *n*-hexane or *i*-octane, Chiu et al had used the Ames test to determine the bacterial mutagenicity of 18 of these compounds (34). One of these chemicals, nitromethane, has a k_e that is not diffusion-controlled (15) and it was found to be non-mutagenic; of the remaining 17, Chiu et al found 10 (~60 percent) to be mutagenic. It was from these 17 nitroaromatics that we chose chemicals for our mutagenicity study.

Our Ames-test results are compared with those of Chiu et al in Table VII for 10 of the nitroaromatics that we have tested. In both studies, only the TA98 and TA100 strains of S. typhimurium were used because these strains have a nitroreductase enzyme that is capable of converting the nitro group to an active intermediate which makes the addition of an external activating enzyme system unnecessary (35). For 8 of the 10 chemicals tested, our results agree with these of Chiu, but for *p*-nitroaniline we observed a positive mutagenic response whereas the response observed by Chiu's group was negative. Also, for *p*-nitrobenzoic acid Chiu observed positive and negative responses for TA98 and TA100, respectively, whereas we observed the reverse strain-mutagenicity dependence. These differences are, perhaps, attributable to the toxicity of these compounds which can produce false-negative responses if corrections for revertant survival are not considered (36). We are attempting to develop a protocol for accurately determining survival since this is especially important in our radiation-mutagenesis studies which we shall now describe.

Table VII. Comparison of the bacterial mutagenicity^a of 10 nitroaromatic compounds as determined with the Ames *Salmonella* bioassay by Chiu *et al*^b and in this work.

<u>Nitro Compound</u>	<u>isomer</u>	<u>Chiu et al</u> ^b		<u>This work</u>	
		<u>TA98</u>	<u>TA100</u>	<u>TA98</u>	<u>TA100</u>
nitrobenzene	-	0	0	0	0
nitrotoluene	p-	0	0	0	0
nitroaniline	o-	0	0	0	0
	m-	+	0	+	0
	p-	0	0	+	0
nitroanisole	o-	+	+	+	+
	m-	0	0	0	0
	p-	+	+	+	+
nitrobenzoic acid	m-	0	+	0	+
	p-	+	0	0	+

(a) Positive and negative mutagenic responses denoted by + and 0, respectively.

(b) Reference (34).

D. Radiation-Induced Bacterial Mutagenesis

Ionizing radiation is the most ubiquitous, most thoroughly studied and yet the most controversial mutagen and carcinogen to which we are exposed (37). Although radiation biology has contributed greatly to our understanding of the gross mutagenic and carcinogenic effects of radiation, a number of questions concerning the basic interactions of radiation with the target biomolecule remain. Since the Ames test has been valuable in identifying chemical mutagens and carcinogens, it appears to be the ideal technique to apply to elucidate the mechanism of radiation-target interaction. With this premise, we have initiated a study of radiation-induced mutagenesis of the Ames Salmonella tester strains.

We know of only three studies of this subject: Ames reported a positive mutagenic response of his tester strains exposed to UV, fast neutrons and X-rays (22); Heddle and Bruce reported a negative mutagenic response of gamma-irradiated Salmonella (38); and most recently, Imray and Macphee reported a positive mutagenic response of gamma-irradiated Salmonella (39).

We ascribe the disparate results of the latter two studies as a reflection of the importance of considering revertant survival in a mutagenicity study which has been discussed by Kaden et al (36) and is demonstrated for two strains of S. typhimurium in Table VIII where our recent radiation-induced mutagenesis results are presented. If one only considers the number of revertants that the radiation induces, it appears that no mutagenic effect is observed. However, if bacterial survival is determined concurrently with reversion frequency, the mutagenicity of the radiation is delineated. For example, TA100 irradiated to a total dose of 3.5 krads produces 215 revertants/plate, which is not significantly greater than the spontaneous reversion frequency of 180 revertants/plate. However, the concurrently conducted viability experiment shows that only 29 percent of the non-mutated bacteria survive this dose, and if we assume that the same survival frequency applies to mutants, then the

Table VIII. Dose dependence of reversion frequency, percent survival and survival-corrected revertants for Salmonella typhimurium strains TA1537 and TA100 irradiated by Co-60 γ radiation at a dose rate of 1.75 krad/min.

<u>Dose</u> <u>(krad)</u>	<u>TA1537</u>			<u>TA100</u>		
	<u>rev/plate</u>	<u>% survival</u>	<u>rev*</u> ^(a)	<u>rev/plate</u>	<u>% survival</u>	<u>rev*</u>
0	10	100	10	180	100	180
0.87	9	71	15	208	100	208
1.7	11	69	16	219	91	241
2.6	7	52	13	247	33	748
3.5	11	40	27	215	29	740

(a) $\text{rev}^* = [(\text{rev/plate})/\% \text{ survival}] \times 100$

reversion frequency is 740 revertants/plate which is four times the spontaneous reversion rate. Consequently, we routinely conduct a viability study with conditions identical to the mutagenicity study except that 10^3 cells/plate are used in the viability experiment compared to 10^8 /plate for reversion frequency.

We have conducted an analogous study of TA98 and found this strain to be considerably more sensitive to radiation. For example, at a dose of 1.7 krad, only 5 percent of the bacteria survive; consequently, we used X-rays at a lower dose rate as the radiation source for mutagenicity studies of this strain. Again radiation-mutagenesis was observed; at a dose of 0.9 krad where 18 percent of the bacteria survive, 33 revertants/plate were produced which yields a survival-corrected mutation frequency that is ninefold greater than the spontaneous reversion frequency of 20 revertants/plate.

In order to establish that the observed radiation-induced mutagenesis was not produced by the formation of radiolytic mutagenic products on the plate substrate (agar) or in the nutrient broth, we added the tester strains to plates and broth that had been irradiated to the same dose and a tenfold greater dose than is used in the mutagenicity studies. No significant mutagenesis was observed in bacteria grown upon the pre-irradiated plates either in the presence or absence of the pre-irradiated broth.

These studies indicate that radiation-induced mutagenesis can be efficaciously monitored using the Ames test which will permit us to approach the question of the role that electrons play in biological systems from a fresh viewpoint. This biological approach will complement our well established physico-chemical approach and lead us to the goal by the route proposed in our accompanying research proposal.

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APPENDIX

I. Publications

- A-1 . . . . . Bakale, G. and Schmidt, W.F., "Effect of an Electric Field on Electron Attachment to SF₆ in Liquid Ethane and Propane", Z. Naturforsch. 36a, 802-806 (1981).
- A-6 . . . . . Bakale, G. and Schmidt, W.F., "The Effect of an Electric Field on Electron Attachment to SF₆ in Non-Polar Liquids", Proc. 7th Int. Conf. Cond. Breakdown Diel. Liq., W.F. Schmidt, Ed., pp. 55-57, W. Berlin, July 27-31, 1981.
- A-9 . . . . . Bakale, G., Beck, G. and Thomas, J.K., "Electron Capture in Water Pools of Reversed Micelles", J. Phys. Chem. 85, 1062-1064 (1981).
- A-12. . . . . Bakale, G., McCreary, R.D. and Gregg, E.C., "Quasifree Electron Attachment to Carcinogens in Liquid Cyclohexane", Cancer Biochem. Biophys. 5, 103-109 (1981).
- A-19. . . . . Hissung, A., Isildar, M., von Sonntag, C. and Witzel, H., "Radiolysis of Aqueous Solutions of Nucleosides Halogenated at the Sugar Moiety", Int. J. Radiat. Biol. 39, 183-193 (1981).
- A-28. . . . . Isildar, M., Schumann, H.N., Schulte-Frohlunde, D. and von Sonntag, C., "γ-Radiolysis of DNA in Oxygenated Aqueous Solutions: Alterations at the Sugar Moiety", ibid. 40, 347-354 (1981).

II. Presentations:

Bakale, G., McCreary, R.D. and Gregg, E.C., "Effect of Electron-Dipole Interaction on the Electron Attachment Rate of Nitroaromatic Solutes in Liquid Isooctane", to be presented at the 30th Annual Scientific Meeting of the Radiation Research Society, Salt Lake City, Utah, April 18-22, 1982.

Isildar, M. and Bakale, G., "Enhancement of Radiation-Induced Mutagenesis by Non-Mutagenic Chemicals", ibid.

III. Other Activities:

Bakale, G., Guest Scientist at the Hahn-Meitner Institut für Kernforschung, West Berlin, Germany, May 1 - June 30, 1981.

Isildar, M., Attended DNA Sequence Analysis Workshop offered by Bethesda Research Laboratories at Case Western Reserve University, Cleveland, Ohio, September 21-23, 1981.