RESEARCH PROGRESS REPORT

100-200000-10000
100-200000-10000
100-200000-10000

MECHANISMS OF INTERACTION OF RADIATION WITH MATTER.

Submitted to:

DEPARTMENT OF ENERGY

Division of Biomedical and Environmental Research

Period: September 1, 1989 to August 31, 1990

RADIATION AND SOLID STATE LABORATORY

NICHOLAS E. GEACINTOV and MARTIN POPE, PROJECT DIRECTORS

New York University

Department of Physics & Chemistry

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED *ps*

TABLE OF CONTENTS

INTRODUCTION.....	1
PART I. PHOTOEMISSION FROM PARTICULATES.....	3
A. Introduction.....	3
B. Pyrene.....	3
C. Photoemission in nitropyrenes.....	13
D. Stability of phthalocyanines under high intensity illumination....	15
E. Nucleic acid base crystals: adenine.....	16
F. Computer simulation of electron emission from particulates into gases.....	17
G. Theoretical study of excitonic ion.....	18
PART II. ELECTROLYSIS AT AN ANTHRACENE/AQUEOUS SOLUTION INTERFACE.....	19
A. Introduction.....	19
B. Organic cation radical reactions at an anthracene/aqueous solution interface.....	20
C. Effect of annealing of anthracene crystals on electrolytic reactions.....	21
PART III. MECHANISMS OF INTERACTIONS OF POLYNUCLEAR AROMATIC COMPOUNDS WITH DNA BASES.....	24
A. Introduction.....	24
B. Overview of methods of approach.....	27
C. Mechanisms of interaction between the pyrenyl and base moieties in BPDE-deoxynucleoside adducts.....	31
D. Fluorescence techniques for distinguishing DNA adducts derived from highly tumorigenic and non-tumorigenic stereoisomers of BPDE..	44

E. Conclusions and future outlook.....	57
LIST OF PUBLICATIONS (01/01/1989 - 8/31/1990).....	60
LIST OF PERSONNEL.....	62

APPENDIX

One preprint

10 reprints

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

INTRODUCTION

This progress report is a summary and update of the research performed under DOE grant FG-02086-ER60405 from September 1, 1989 to August 31, 1990. Part I deals with mechanisms of photoemission from organic particulates, theoretical studies of the photoemission of electrons into atmospheres containing scavenger molecules, and theoretical studies of the possible existence of excitonic ions. Part II deals with the mechanisms of electrolytic reactions which occur at solid anthracene/aqueous electrolyte interfaces. Part III describes our most recent results on the physico-chemical interactions of mutagenic and carcinogenic polycyclic aromatic hydrocarbon (PAH) derivatives with nucleic acids.

Some of the highlights of our recent research results are summarized as follows:

I. Water molecules lower the ionization potentials of adenine and other nucleic acid bases.

Solid adenine (one of the four DNA bases) is characterized by two ionization potentials (6.2 eV and 7.1 eV). We have resolved this long-standing problem by discovering that the upper value is characteristic of pure dry adenine, crystal while the lower value is attributed to adenine whose surface molecules are surrounded by water. This lowering of the ionization potential by H_2O is attributed to the ability of a solvation layer to re-orient itself on time scales of 10^{-13} to 10^{-12} s, the approximate lifetime of a putative pre-ionizing state of adenine.

II. Exciton levels, band gaps, and photoemission mechanisms in solid pyrene and nitropyrene derivatives.

These have been characterized using the Millikan-Pope-Arnold apparatus. In the 1- and 2-nitropyrene solids, the collisions of mobile triplet excitons with

trapped electrons contribute to a double-quantum photoemission mechanism. In solid pyrene, the interactions of thermally activated singlet excimer states with each other and with trapped electrons are involved.

III. The physico-chemical interactions between tumorigenic benzo[a]pyrene and pyrene derivatives and DNA bases have been characterized to distinguish between tumorigenic and non-tumorigenic metabolic derivatives bound to DNA.

Conformation-dependent photoelectron transfer interactions between the polycyclic aromatic residues and nucleic acid bases have been studied. The efficiencies of these processes depend on the local dielectric constant and the structures of the complexes. The photophysical characteristics of these processes can be used to distinguish between different adduct conformations in DNA adducts, derived from mutagenic and non-mutagenic isomers, especially by fluorescence techniques.

IV. Synthesis and characterization of stereospecific and base-specific carcinogen-DNA adducts.

A most significant development has been our recent success in synthesizing high yields of small oligonucleotides of defined base composition and sequence modified in a chemically and sterically specific manner at a single site in short chain nucleic acids (9-11 base pairs long). Studies with these oligonucleotides with selected mutagens and carcinogens are beginning to yield exciting new insights into relationships between molecular structures of mutagen-DNA complexes and biological effects.

Finally, a list of publications describing work supported in whole or in part by this DOE grant is appended.

PART I PHOTOEMISSION FROM PARTICULATES.

A. INTRODUCTION.

The important role of particulates in the physics, chemistry and biology of the environmental hazards created by the production of energy from both fossil and nuclear sources is beyond question. Particulates in their most inert form act as conveyors of more pernicious agents into the lung recesses. An example of this would be the deposition of radon disintegration products on dust which, when inhaled, lodges in the lung sacs. Particulates can also potentiate the effect of radiation damage by virtue of their composition. Thus, a particulate that contains a radionuclide and a tumorigenic agent is clearly a greater menace; cigarette smoke would fall in this category. As part of our basic particulate research program, we have studied a wide variety of materials. These include pyrene, adenine, and graphite.

B. PYRENE.

Pyrene is a polycyclic aromatic hydrocarbon in which the unit cell of the crystal contains pairs of parallel molecules instead of single molecules as are found in anthracene at atmospheric pressure and at room temperature. As a result, there are unusual electronic states in this material, such as excimer excitons [1]. An excimer is an electronically excited dimer that is stable only in the excited state, but decays dissociatively into a pair of monomeric ground state molecules with the emission of a characteristic structureless fluorescence identified as excimer fluorescence. Excimers often appear at defect sites in monomeric crystals [2], and in polymers that contain pendant PAH groups that can line up parallel to each other. The appearance of excimer emission in a monomeric crystal is diagnostic of positional disorder.

It is known that excimer excitons can migrate through the pyrene lattice at room temperature. It is also known that two excitons can migrate to each other and react, producing a highly excited state that will photoemit an electron. This

process is referred to as a double quantum external photoelectric effect (DQEPE). This process can often be used to help understand the mechanism of the photoionization process.

Some open questions regarding the excimer excitons are the following:

- 1) In what electronic state does exciton migration take place in pyrene?
- 2) In a paper by Klopffer and Bauser, [3] involving the quenching of the pyrene crystal excimer fluorescence by a coronene dopant, an excimer diffusion coefficient of about $3 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ was deduced, from which a formal bimolecular rate constant γ_{KB} for quenching can be calculated as [4]

$$\gamma_{KB} = 4 \pi D \langle R \rangle$$

where D is the diffusion coefficient and $\langle R \rangle$ is an effective radius of capture. According to Zeiri et al [5], $\langle R \rangle = 30 \text{ \AA}$, giving a value of $\gamma_{KB} = 1 \times 10^{-10} \text{ cm}^3 \text{ s}^{-1}$. However, for the quenching of one pyrene excimer by another excimer, the value γ was calculated to be $10^{-14} \text{ cm}^3 \text{ s}^{-1}$. The explanation of this discrepancy is a glaring open question.

- 3) It has been found that photoemission can take place in pyrene as a result of the interaction of two excitons. In what electronic states are these excitons reacting with one another?

- 4) What is the role of surface defects in the photoemission process?

In Fig. 1 is shown the most complete action spectrum for photoemission ever made for an organic crystal. Despite the fact that the intrinsic photoemission threshold for pyrene crystal is 5.8 eV, photoemission is observed to start at 3.4 eV [6]. For incident photon energies $< 5.8 \text{ eV}$, the photoemission process requires the participation of two metastable excited states, at least one of which is mobile. Thus, for photon energies $< 3.95 \text{ eV}$, the photoemission yield (Y) varies as the square of the light intensity (I^2), which is indicative of a simple bimolecular reaction. For a photon energy of 3.95 eV, the yield $Y \propto I^{1.5}$. Furthermore,

it was possible, using our technique, to calculate the combined energy of the two metastable states that were responsible for the photoemission.

Before proceeding, it is important to define a term to be referred to as the equivalent energy E_{eq} . When excited states interact with each other, it is generally not known what the precise energies of all the excited states are. Thus, the absorption of a photon of a given energy will excite a state that can relax to a lower-lying state that participates in an exciton fusion process. The energy of this lower-lying state is generally not known. The known quantity in the case of photoemission, is the maximum kinetic energy of the emitted electron which is an experimentally realizable quantity. Assume now a case in which photoemission is produced as a result of the absorption of only one photon of known energy that exceeds the threshold for photoemission I_C ; this is called the single quantum external photoelectric effect or SQEPE. The maximum kinetic energy of the emitted electron is then $(E_{ph} - I_C) = K.E._{max}$. Now consider the case of photoemission that is produced by a DQEPE, in which the energy of the reactants are unknown. The maximum kinetic energy of the emitted electron in the DQEPE can be measured, and may be referred to as $K.E'_{max}$. A series of SQEPE photoemission experiments are then carried out, and by suitable interpolation, it is possible to find a SQEPE for which the maximum kinetic energy of the electron is $K.E._{max}$, identical to the value found for the DQEPE. The photon energy for the SQEPE that produces the value $K.E._{max}$ is referred to as the equivalent energy E_{eq} generated by the fusion of the two states in the DQEPE. It was found that for many (> 15) pyrene crystallite samples, for incident photon energies of 3.4 eV and 3.7 eV, the combined energy of the two metastable states was independent of the photon energy and yielded an E_{eq} that was either 5.9 eV or 6.2 eV. A question to be answered is why are there two values for E_{eq} . For the 3.95 eV photon energy, for many pyrene samples (> 15) the E_{eq} was either 5.9, 6.3 or 6.8 eV. Another question is why are there three values for the combined energy of the interacting states.

PYRENE

Photoemission

Yield of photoemission from pyrene crystal vs Energy of incident photons (Yield is in electrons/photon in a.u.)

PART I

FIGURE 1

page 5a

One of the important experiments that was carried out was to compare the photoemissive yield produced by a single quantum (SQEPE) of 6.2 eV, with the yield obtained from a DQEPE for which the equivalent energy was also 6.2 eV. Schematically, the processes may be described as follows: For the single quantum process:

While in the double quantum process:

where P^{**} is a molecularly excited state in the crystal, γ is a bimolecular rate constant, η is the efficiency of producing photoelectrons, $(1 - \eta)$ would be a parallel path for the decay of P^{**} to a lower lying state. In the case of the quenching of the fluorescence in pyrene as a result of an excimer-excimer reaction, equation (2) would be the appropriate description, with $(1 - \eta)$ being the efficiency of the quenching process.

In the two schemes just outlined, it may be seen that if the photoemission current is measured on the same crystal for the single quantum and double quantum processes, then the relative magnitude of these currents would be determined only by the rate of producing P^{**} in each process, if P^{**} is assumed to be virtually the same final state in equations (1) and (2). The rate of producing P^{**} in the single quantum process is $k_1 I_1$ where k_1 , and I_1 are, respectively, the absorption coefficient and light intensity of the 6.2 eV light. The rate of producing P^{**} in the

double quantum process is:

$$\begin{aligned}\gamma [X^*]^2 &= \gamma [X_0 e^{-E/kT}]^2 = \\ &= \gamma e^{-2E/kT} [k_2 I_2 \tau_2]^2 = \gamma' [k_1 I_2 \tau_2]^2\end{aligned}\quad (3)$$

where γ is the bimolecular rate constant for the encounter of two X^* excimers, γ' is the effective bimolecular rate constant, E is the energy required to excite X_0 to X^* ; k_2, I_2 are the absorption coefficient and light intensity of the double quantum light, and τ_2 is the lifetime of the ground state excimer. All of the necessary data are available, except for the value γ' . Thus, if i_1 is the photoemission current in the single quantum process, and i_2 is the emission current in the double quantum process, then

$$\frac{i_1}{i_2} = \frac{k_1 I_1}{\gamma' [k_2 I_2 \tau_2]^2}$$

and

$$\gamma' = \frac{k_1 I_1}{[k_2 I_2 \tau_2]^2} \times \frac{i_2}{i_1} \quad (4)$$

The data are as follows:

$\frac{6.2 \text{ eV}}{I_1 = 3.3 \times 10^{12} \text{ cm}^{-2} \text{ s}^{-1}}$	$\frac{3.4 \text{ eV}}{I_2 = 1.2 \times 10^{17} \text{ cm}^{-2} \text{ s}^{-1}}$
$k_1 = 8.7 \times 10^5 \text{ cm}^{-1}$	$k_2 = 1.2 \times 10^{-5} \text{ cm}^{-1}$
$i_1 = 1.5 \times 10^{11} \text{ (arb. units)}$	$i_2 = 1.7 \times 10^9 \text{ (arb. units)}$
	$\tau_2 = 10^{-7} \text{ s}$
$\gamma'(\text{calc}) = 1.7 \times 10^{-14} \text{ cm}^3 \text{ s}^{-1}$	

Fortunately, the value of γ' has been measured by Inoue et al. [7] for the excimer fluorescence quenching as described by equation (1); its value is

$$\gamma' \text{ (fluorescence quenching)} = 9 \times 10^{-15} \text{ cm}^3 \text{ s}^{-1} \quad (5)$$

Within experimental error, these values are the same. This is a remarkable result. Some conjectures may be made at this point. Recall, that a key assumption made concerning the state P^{**} in equations (1) and (2) is that this state is virtually the same state regardless of whether the excitation is from the absorption of a single photon or from the fusion of two lower energy excited states. These lower energy excited states at 3.1 eV could be excited excimers [8], or monomers [9]. Since P^{**} produced by the SQEPE is certainly a monomer, having been excited from a monomeric state, it seems to us that the X^* states in equation (2) should also be monomeric. This conclusion favors the mechanism sponsored by Seyfang et al [9]. However, the excited dimer at 3.1 eV that is hypothesized by Matsui's group [8] is presumed to have an intermolecular separation that is almost the same as that of the ground state dimer, and this would correspond closely to the intermolecular separation for Seyfang et al [9]. It may very well be the case that it is impossible to distinguish between the Matsui et al [8] hypothesis, and that of Seyfang et al [9].

In any event, it is clear that the exciton that actually moves in the pyrene lattice is not the relaxed excimer, but is a thermally excited excimer with an intermolecular separation that closely resembles that of the ground states paired molecules in the crystal lattice. This answers the first of the open questions in the pyrene system.

This brings us to the second open question, namely, the large difference between $\gamma_{KB} = 10^{-10} \text{ cm}^3 \text{ s}^{-1}$ obtained by impurity quenching of the excimer, and $\gamma = 10^{-14} \text{ cm}^3 \text{ s}^{-1}$ obtained by the exciton-exciton quenching, and also by the photoemission process. For enlightenment of this problem, examine equation (3), where the effective bimolecular rate constant was defined as

$$\gamma' = \gamma e^{-2E/kT} \quad (6)$$

where E is the energy required to excite the ground state excimer X_0 to the state X^* ; this is 650 cm^{-1} , [9] so $2E = 1300 \text{ cm}^{-1}$. It therefore appears that $\gamma = 10^{-11} \text{ cm}^3 \text{ s}^{-1}$. This is within a factor of 10 of the value of $\gamma_{KB} = 10^{-10} \text{ cm}^3 \text{ s}^{-1}$ that may be calculated from the work of Klöpffer and Bauser [3]. This goes a long way in explaining the large difference between γ' and γ_{KB} , which was a mystery. Another conclusion that can be made is that the fluorescence quenching process is also an $X^* + X^*$ process, or that the value of γ' for the $X_0 + X^*$ process is the same as for the $X^* + X^*$ process.

Now consider the third open question, that of the states of the excimer excitons that can react with each other to produce photoemission. Recall, that when light of energy 3.4 eV was used to excite the pyrene in a DQEPE, some crystals gave a value $E_q = 6.2 \text{ eV}$. How can one account for these values of E_q , and why do some crystals give different values of E_q than others?

The energy of the relaxed excimer exciton in pyrene is 2.7 eV. Therefore, two relaxed excimer excitons have a combined energy of $2 \times 2.7 = 5.4 \text{ eV}$ which is insufficient to produce photoemission, so this process would not be detected in our experiment even if it took place. The collision of a relaxed excimer exciton at 2.7 eV and an excited excimer exciton at 3.1 eV produces a combined energy of $2.7 + 3.1 = 5.8 \text{ eV}$, which corresponds within experimental error to the ionization threshold value I_c . The quantum yield at the threshold value I_c is very low. If it were easily possible to produce a thermally excited excimer exciton, then the quantum yield of photoemission would be greatly improved. This is the case with pyrene crystal. The broad fluorescence spectrum that is characteristic of the pyrene excimer in the crystal that should easily be possible for slightly excited excimers to become involved in the DQEPE. For an expenditure of about $1/2kT$ in

thermal energy, an excimer state at 2.8 eV can be excited. This state would be more efficient in the DQEPE than the 2.7 eV state and we find it reasonable to use this energy of 2.8 eV to rationalize our results. The collision of a state at 2.8 eV with one at 3.1 eV would yield the observed value of $E_q = 5.9$ eV. The collision of two excited excimer excitons would yield a combined energy of $2 \times 3.1 = 6.2 \pm 0.1$, which is the second observed energy. These two excimer-excimer processes would show an I^2 dependence of the yield Y , as is observed.

As for the 3.95 eV result, a conduction electron lying at 4.0 eV reacting with a relaxed excimer exciton at 2.8 eV would give a combined energy of 6.8 eV, as is observed. Moreover, the light intensity dependence of the yield in this case should be $I^{1.5}$, as is observed. For photon energies approaching 5 eV, the light intensity dependence of the yield approaches unity.

These results in which different but distinct equivalent energies are observed for different crystals using the same photon energies are attributed to crystal defects introduced during the preparation of the pyrene crystallites. The rationalization of this hypothesis will now be discussed.

Effect of Annealing on Equivalent Energy

The different groups of crystal results are assumed to be due to the presence of specific types of crystal defects. This difference in behavior from crystal to crystal was also observed by Seyfang et al [9] in their luminescence experiments. Crystal differences therefore seem to be common. Although there may be subtle effects that are not yet appreciated, it would appear to us that an annealed crystal should show the lower energy (5.9 eV) result for the following reason: Crystal defects in crystals like pyrene, in which sandwich pairs of molecules are situated at each lattice site, consist of monomers. The ionization energy in a distorted surface region containing monomers is likely to be slightly higher than in an undisturbed region.

In more than 40 experiments carried out on annealed and unannealed (crushed) crystals, it was found that among the annealed crystals, the 5.9 eV value was more prevalent. Also, the value of I_c was determined for some of the crystals giving the 5.9 eV for the DQEPE, and compared with the I_c obtained for the crushed unannealed crystals. It was found that the value of I_c for the unannealed crystals was 5.9 eV, but this is within the experimental error range of the experiment, so no firm conclusion can be drawn at this time. But the photoemission yield is proportional to the cube of the maximum kinetic energy of the emitted electrons [4], which would favor the process involving the higher energy excited excimer excitons by a factor of 37. For this reason, the DQEPE that gives the higher total energy would be the favored process in the unannealed crystal, and this would be the excited excimer-excimer reaction yielding 6.2 eV.

Light-Intensity Dependence of Yield at 5 eV

Our recent results have therefore provided reasonable explanations for the past puzzling observations. There was one inconclusive observation that was made in our laboratory in which a quantum yield $Y \propto I$ characteristic of a SQEPE was seen when the incident photon energy was 5.0 eV. Since $I_c = 5.8$ eV, it is not possible for there to be a SQEPE involving pure pyrene. This experiment was therefore repeated, and is described here.

In repeating the experiments carried out at photon energies of 5 eV, it was found that although a linear light intensity dependence was observed, there were also infrequent observations of a $Y \propto I^{1.5}$ dependence. It was also found that at a photon energy of 4.7 eV, $Y \propto I$ in many experiments. Furthermore, the yield at 5 eV and at 4.7 eV was sufficiently low to make it impossible to vary the light intensity by more than a factor of three. With this limitation in mind additional experiments revealed the following: For $4.0 < E_{ph} < 4.7$ eV, it was always the case that $Y \propto I^{1.5}$. For $4.7 < E_{ph}$, then at times, $Y \propto I$.

One obvious avenue to check was the possibility of surface or bulk contamina-

tion of the sample by an impurity of lower ionization energy than that of pyrene. A direct way to check this is to measure the maximum kinetic energy ($KE_{\max}$) of the emitted electron as a function of incident photon energy. The $KE_{\max}$ of electrons emitted from an impurity would scale linearly with incident photon energy. This was done, and it was found that $KE_{\max}$ increased by 0.3 eV when the incident photon energy was varied from 4.7 to 5.0 eV. Here again, in view of the error limits of ± 0.1 eV, a conclusion that an impurity was present can only be made tentatively.

To further test the hypothesis that an unknown impurity was present, a pyrene sample that was made purer was studied, and prepared in a more careful manner. The incidence of cases where $Y \propto I$ dropped drastically, lending greater credence to the impurity hypothesis. A possible candidate for the surface impurity might be the ubiquitous carbon.

As for the $Y \propto I^{1.5}$ response, it was decided to check the proposed mechanism of an electron + exciton interaction by measuring the dependence of Y on I when first the electron concentration was varied at constant excimer concentration, and then vice-versa.

The following approach was therefore used. Doped pyrene crystals were obtained from G. Sloan at the duPont Laboratories (0.1% perylene in pyrene) in which the blue fluorescence of pyrene was essentially quenched in favor of the yellow fluorescence of the perylene in pyrene. This implied that the relaxed excimer lifetime was determined by the rate of capture by the perylene dopant. These doped crystals showed no double quantum external photoelectric effect (DQEPE) at a photon energy of 3.4 eV, and a weak almost non-existent DQEPE at 4.6 and 5.0 eV. The absence of a DQEPE in the doped pyrene (excimer excitons are quenched) confirmed the involvement of excimer excitons at 3.4 eV in the neat crystal, and also showed that at 4.6 and 5 eV there was also considerable but perhaps not exclusive exciton involvement.

A further experiment was carried out in which two co-linear beams of light, one at 5 eV and the other (at five times higher intensity) at 3.4 eV, were shone on the doped crystal. It was found that the photoemission yield varied linearly with the light intensity at 3.4 eV. Since the 3.4 eV light was ineffective in producing any DQEPE, and the 5 eV photon at low light intensity could only be producing electrons (free or trapped) as effective partners for the 3.4 eV light, it appears that the process at a photon energy of 5 eV for which $Y \propto I^{1.5}$ is due to the interaction of a photon with a free or trapped carrier.

Additional experiments were carried out in which the 5 eV light intensity at constant 3.4 eV light intensity on the doped crystal. As expected, the yield was found to obey an $I^{0.5}$ light intensity dependence verifying the role of electrons in this process.

C. PHOTOEMISSION IN NITROPYRENES

As an extension of our work with pyrene, we started a series of photoemission experiments with the more mutagenic nitropyrenes, whose electronic states in the solid are essentially unknown. There are three distinct isomers of this compound: the formulas for which are shown below:

The 2-NP is quite expensive in its pure state, so we had a sample of only 10 mg. The dilute solution absorption spectra of 2-NP is known, and is similar to that of pyrene. This is attributed to the attachment of the NO_2 group to a nodal plane carbon atom. The threshold ionization energy for 2-NP was found to be 5.8 eV, the same as for pyrene. A DQEPE was found for this material for which the light inten-

sity dependence of the yield was $Y \propto I^{1.5}$. This effect was seen when the incident light energy reached 3.7 eV. From this, we conclude that $E_g \sim 3.7$ eV.

In addition, there was a DQEPE in which $Y \propto I^2$ for light of energy $E_{ph} = 3.4$ eV implying that the singlet state lies below 3.4 eV but above 2.9 eV.

It also implies that the singlet lifetime is long enough to produce a DQEPE. We guess that its lifetime at room temperature is longer than 10^{-10} s. Based on the involvement of an exciton with the electron, the exciton energy is calculated to be 2.1 eV. This cannot be a singlet exciton because the color of the crystal would then be green, whereas it is off-white. The 2.1 eV exciton was judged to be a triplet exciton, since the transition from singlet to triplet state is so efficient and rapid that there is no fluorescence at room temperature. This conclusion was verified by measuring the phosphorescence of 2-NP in a low-temperature glass. The peak in the phosphorescence intensity occurred at an energy of 2.1 eV.

In analogy with the solution spectra, it is therefore predicted that the singlet state of 2-NP will lie close to that of pyrene, at 3.3 eV. An interesting divergence from the similarity between 2-NP and pyrene is in the value of the band gap E_g . For pyrene, it is 4.1 eV whereas for 2-NP, it is less than but close to 3.7 eV. The summary of energy levels is therefore

	<u>1NP</u>	<u>2-NP</u>
I_c	5.6 eV	5.8 eV
E_g	3.4 eV	3.7 eV
E_s	---	(~ 3.3 eV)
E_T	2.2 eV	2.1 eV

One possible reason for the differences in the values of E_g between pyrene and 2-NP is that the electronic excitations all involve the π electrons in the ring. This also holds for the photoemission. However, in the intrinsic carrier generation process, the value of E_g is determined not only by the ionization energy of the electron system, but the electron affinity A_g of the 2NP. The electron affinity (A_g) of the NO_2 group is likely to be greater than that of the ring system; a guess would be that A_g of 2NP is ~ 0.4 eV larger than that of pyrene.

As for the 1-NP, it was found that $I_c = 5.6$ eV, and $E_g = 3.4$ eV. There was no DQEPE for energies less than 3.1 eV. The DQEPE observed at $E_{ph} = 3.4$ eV had a light intensity dependence $Y \propto I^{1.5}$, implying the involvement of an electron. Since the DQEPE commenced at $E_{ph} = 3.4$ eV, it was concluded that $E_g \sim 3.4$ eV, and that the energy of the triplet exciton for 1-NP lay at 2.2 eV. This was confirmed by an measurement of the phosphorescence from a low temperature glass containing 1NP.

Since it was possible to observe a DQEPE for 2NP for which the yield $Y \propto I^2$ for light of energy $E_{ph} = 3.4$ eV, implying the involvement of two singlet excitons, we infer that the singlet exciton lifetime is 2-NP must be greater than that of 1-NP. It is also reasonable to guess that the lifetime will be larger than 10^{-10} s and the energy of the singlet exciton E_s must be greater than 2.9 eV, in order that two singlets provide enough energy to overcome the threshold ionization energy $I_c = 5.8$ eV. Most likely, E_s will be about 3.3 eV, the same as for pyrene.

D. STABILITY OF PHTHALOCYANINES UNDER HIGH INTENSITY ILLUMINATION

As a preliminary step to studying time-resolved photoemission from metal-free phthalocyanine ($\beta\text{-H}_2\text{P}_c$), it was necessary to determine whether the material could withstand the high intensities generated by the ps laser.

Instead of $\beta\text{-H}_2\text{P}_c$, we used CuPc, which has the same optical characteristics, and found that the 2.37 eV light at its greatest intensity (4.8×10^{29} quantum cm^{-2}

s⁻¹) caused the particle to disintegrate. By varying the intensity, it was found that when the intensity was in the range of $<3 \times 10^{28}$ quanta cm⁻² s⁻¹ at 2.33 eV, the particle was stable.

An interesting observation was that of a DQEPE at 2.33 eV. Since the singlet state of CuPc lies at about 1.8 eV, singlet exciton involvement is ruled out. There are two disputed values [10] for I_c for CuPc: one is 4.6 eV and the other is 5.0 eV. Since two photons provide 4.66 eV of energy, it is clear that the 4.6 eV value is correct. It also follows that we have observed a 2-photon ionization process, which is the first we have observed in our MPA apparatus.

E. NUCLEIC ACID BASE CRYSTALS: ADENINE.

Electron-transfer reactions are of fundamental importance in biological processes since these can bring about structural changes, and are also involved in the storage and release of energy. The reaction between a polycyclic aromatic hydrocarbon (PAH) and DNA can lead to mutagenesis, but the mechanism of this reaction is not known. It is known that exciplexes between PAH compounds and DNA can form and that this complexation could be central to the action of the PAH compounds. Exciplex formation is sensitive to the electron donating and accepting tendencies of the reacting pair, and it is extremely helpful to know the ionization energies and other electronic properties of both DNA and the PAH.

The DNA contains as constituents, among other structures, the nucleic acid bases adenine, cytosine, guanine and thymine. As a prelude to studying DNA itself, it is instructive to determine the electronic properties of the bases, and even more to the point, to determine the effect of water on their electronic properties. This has been done on adenine [Appendix A] and a most interesting result was obtained. The ionization energy of solid adenine is significantly reduced (from 7.1 to 6.2 eV) by the presence of water on the surface. This is due to the greater polarizability of water relative to that of the underlying adenine. However, when a pre-ionizing surface adenine molecule covered by water molecules is electronically excited to an

energy of 6.2 eV, the polarization energy must be in place before the electron leaves the crystal. It therefore appears that the electronic orbital of the excited molecule is sufficiently extended to expose the water molecules to the positively charged adenine molecular core, which then proceeds to polarize the water molecules, and eventually, the electron leaves the crystal. This process takes time, estimated to be about 6×10^{-13} s. This is an important finding, and a fuller discussion is contained in the paper in Appendix A.

F. COMPUTER SIMULATION OF ELECTRON EMISSION FROM PARTICULATES INTO GASES.

The program to simulate electron photoemission from particulates into gases, using the complex geometry of the MPA apparatus is continuing. Some of the interesting results are summarized below.

a). The higher the initial electron velocity, the larger the relative importance of diffusion, as compared with the drift introduced by the external electric field. The emitted electron executes a nearly isotropic random walk which becomes increasingly more biased by the external electric field as the electron thermalizes. This result provides support for a similar major assumption made by Altwegg et al [11] in the derivation of their expression relating the photoemission yield to the electronic properties of the crystal.

b). When scavengers are present, the emitted electrons are captured with an efficiency that increases as the electron slows down. When the electron is scavenged, it is instantly thermalized, and becomes more sensitive to the external electric field. If the electron is far out at the time of capture, by a scavenger, it has an increased probability of escape, but if it is captured by a scavenger near the particle, it has an increased probability of returning to the particle. The result provides support for another major assumption made by Altwegg et al [11] in

the derivation of their expression.

c). At reduced pressure, the probability of escape increases rapidly since the effective diffusion coefficient expands the diffusion sphere before the external electric field begins to be important. This result is in agreement with the experimental result [11] on the increase in yield as the ambient pressure is reduced.

d). For fixed charge on the particle, the probability of escape at first increases and then decreases with increasing escape velocity, leveling off at values above 0.5 eV. The most important single factor is the probability of recapture during the first few collisions since the emitting sphere occupies almost half the available space. This result has not yet been observed experimentally and represents a challenge to the experimenter.

G. THEORETICAL STUDY OF THE EXCITONIC ION.

In several previous papers [12, 13, 14] from this laboratory, a previously unobserved electronic state consisting of a charge-transfer exciton (hole-electron pair) and an electron hole or excitonic ion was proposed to explain some photoemission results from tetracene crystal. Since this state has not yet been observed by anyone else, a theoretical study was undertaken to see if such a state had an intrinsic instability.

In a paper that was published by Munn et al, [15] it was found that the energy of a configuration consisting of a hole-electron pair and a free electron oscillated as the electron approached the positive end of the hole-electron pair. In this first-order treatment, it was found that the reaction was slightly repulsive at all distances, but there were metastable states close to the hole-electron pair in which an electron could be trapped for a long enough time to participate in an Auger ionization [see Appendix B].

In a more refined quantum treatment on which the unique properties of the surface region are taken into consideration, it may turn out that the excitonic ion

has some stable configuration.

PART II ELECTROLYSIS AT AN ANTHRACENE/AQUEOUS SOLUTION INTERFACE.

A. INTRODUCTION.

In many locations across the U.S., accumulations of polyhalogenated hydrocarbons (PCB's) have been uncovered. These materials are chemically stable, and indiscriminate dumping over many years now presents us with a major environmental problem. The PCB's have a harmful effect on animal life, particularly fish, since the dumping almost invariably ends up in a river or ocean. The partially oxygenated products, called dioxins are particularly carcinogenic.

The most commonly used method for destroying PCB's is by incineration. It appears that in this process, some dioxins are released, and this is a cause for concern. Another technique that is being explored is that of electrolytic oxidation and/or reduction, which effectively destroys the PCB's without releasing dioxins [16]. We believe that this technique has great merit, and should be thoroughly explored. One major problem facing those with the responsibility of ridding river beds of the accumulation of PCB's is the great cost that is involved in physically removing this material, and the serious increase in the concentration of PCB's in the river that would follow upon the disruption of the river bottom. In our opinion, it should be possible to destroy the PCB's in situ, by the use of extensive grids of electrodes inserted into the river bottom. The passage of large currents through these electrodes should generate oxidizing and reducing conditions that are conducive to the safe destruction of PCB's, with a minimum of harmful side effects on animal and plant life in the river.

Our experience with the use of anthracene as an electrode in electrolytic

reactions with aqueous systems has shown us that oxidation of the anthracene in pure water is a facile reaction, producing a variety of oxidation products, leading ultimately to the destruction of the anthracene molecule. We have also found that when anions are present in solutions (and also cations like NH_4^+), these anions can be made to discharge at the anthracene surface, and to form derivatives of anthracene. Thus, NO_3^- and NO_2^- ions both end up as nitroanthracene derivatives.

B. ORGANIC CATION RADICAL REACTIONS AT AN ANTHRACENE/AQUEOUS SOLUTION INTERFACE

Many years ago, we discovered that insulating organic crystals could be made conductive by providing them with injecting contacts [4]. These contacts consisted of oxidizing and reducing aqueous electrolytes and they opened up an entire field of study of the electronic processes that go on inside organic crystals [17]. More recently, we have become interested in the chemical reactions that take place at the crystal/solution interface as a result of electrolytic action. We have found a large variety of oxidation products on the surface of the crystal and there are also products formed in solution. We expect that in many cases, the heterogeneous reactions between the radical cations that carry the current in the crystal and the ions that are present in aqueous solution parallel the degradative reactions that take place in biological systems that are subjected to high energy radiation in the presence of water and/or oxygen. An important reactive intermediate in both cases seems to be the OH radical. So far, we have only indirect evidence for the generation of the OH radical as a separate entity. This consists of the finding that H_2O_2 and O_2 are generated during the course of the electrolysis in the absence of ambient oxygen [18].

Another important finding is that when atmospheric oxygen is permitted to permeate the aqueous solutions, there was a decrease in the concentration of surface reaction products per Coulomb of charge flowing in the anthracene crystal used as an

electrode. The mechanism of action of the ambient O_2 is not known. However, many years ago, in their unsuccessful attempt to detect the production of oxygen as a result of the electrolysis of water with an anthracene crystal, Jarnagin et al [19] found that ambient O_2 was consumed during electrolysis, and that water-soluble decomposition products were produced. The production of water-soluble species, which we do not at present analyze for, are not included in our analysis, this would explain the reduction in the yield of insoluble surface oxidation products.

During this period we tried to determine whether OH radicals are produced during electrolysis, and whether singlet O_2 is involved in the reaction of ambient O_2 with the anthracene surface. The electrolysis experiment was carried out in the laboratory of Dr. Ahsan Khan, who is a noted expert in the generation and detection of singlet oxygen.

It was known at the outset of this experiment that the amount of products formed during electrolysis might be below the level of detectability in the apparatus, and unfortunately, this turned out to be the case.

C. EFFECT OF ANNEALING OF ANTHRACENE CRYSTALS ON ELECTROLYTIC REACTIONS.

In our last report, dealing with the reaction of NO_2^- and NO_3^- with the anthracene radical cation, as a result of which the anthracene surface was nitrated [20], we presented evidence that the nitration involved the simultaneous participation of two holes and that this took place at a crystal defect. The evidence consisted of the observation that the nitration reaction was competitive with the production of the dimeric molecule bianthranyl, and also depended on the square of the current density. Thus, the rationale was that since bianthranyl had an absolute steric requirement for the participation of two adjoining sites in order to form the dimer, and since its production also depended on the square of the current density, therefore the competitive production of nitroanthracene also depended on the participation of the same type of adjoining crystal sites as was used by bianthranyl during

its formation, most likely at defect sites. In order to test this hypothesis, we set up a procedure for reducing the number of emergent dislocation by annealing at 170°C for 18 hours.

As is shown in Appendix C, there is a considerable drop in the efficiency of producing 9NA and of BA after annealing. This provides support to the original hypothesis concerning the role of crystal surface defects in aligning the anthracene molecules so that the reactive intermediates to the formation of 9NA could be generated.

This work has been published in the Journal of Physical Chemistry, 92 2506 (1988).

REFERENCES FOR PART I AND PART II

1. D. Fisher, G. Naundorf and W. Klopffer, *Naturforsch.*, 28, 973 (1973).
2. P.F. Jones and M.J. Nicol, *Chem. Phys.*, 43, 3759 (1965).
3. W. Klopffer and H. Bauser, *Chem. Phys. Lett.*, 6 279 (1970).
4. See M. Pope and C.E. Swenberg, Electronic Processes in Organic Crystals Oxford Press, New York, 1982.
5. L. Zeiri, G. Berkovic, and Z. Ludmer, *Chem. Phys. Lett.*, 147 279 (1988).
6. M. Zielinski and C.S. Sheu (Laboratory Report).
7. A. Inoue, K. Yoshihara, and S. Nagakura, *Bull. Chem. Soc. Japan*, 45 1973 (1972)
8. A. Matsui, M. Jemura and H. Nishimura, *J. Lumin.*, 24/25 445 (1981).
9. R. Seyfang, H. Port and H.C. Wolf, *J. Lumin.*, 42, 133 (1988).
10. S.X. Chen, K. Seki, H. Inokuchi, T.R. Shi, and R.Y. Qian, *Bull. Chem. Soc. Japan*, 6 2565 (1983).
11. L. Altwegg, M. Pope and Wm. Y. Fowlkes, *Chem. Phys.*, 86 471 (1984).
12. M. Pope, J. Burgos and J. Giachino, *J. Chem. Phys.*, 43, 3367 (1965).
13. S. Arnold, M. Pope and T.K.T. Hsieh, *Phys. Sta: Sol.(b)*, 94, 263(1979).

14. L. Altwegg, M. Pope and S. Arnold, Wm. Y. Fowlkes, and M.A. El Hamamsy, Rev. Sci. Instr., 53, 332 (1982).
15. R.W. Munn, W.G. Mabbott, and M. Pope, Chem. Phys. (to be published).
16. H. Sugimoto, S. Matsumoto, and D.T. Sawyer, Environ. Sci. Technol. 22, 1182 (1988)]
17. H. Gerischer and F. Willig, Topics in Current Chemistry, 61 315 (1976).
18. M. Pope and K. Slotnick, J. Phys. Chem., 86 1923 (1982).
19. R.C. Jarnagin, J. Gilliland Jr., J.S. Kim and M. Silver, J. Chem. Phys. 39 373 (1963).
20. B. Leong, M. Pope and J. Steigman, J. Chem. Phys, 92 2506 (1988).
21. V.M. Kenkre and D. Schmid, Chem. Phys. Lett., 94 603 (1982).
22. J.M.G. Marinho and V.R. Pereira, J. Lumin., 40 & 41 673 (1988).
23. B.I. Greene and R.R. Millar, Phys. Rev. Lett., 55 1331 (1985).

Part III

A. INTRODUCTION

a. DNA damage and biological end points.

Ionizing radiation and bulky polynuclear aromatic hydrocarbon compounds (PNA) cause DNA damage which can lead to a complete loss of DNA replication or cell death, mutations, and cancer. In living cells, DNA is either in contact with, or in close proximity to oxygenated aqueous environments. Thus ionizing radiation produces DNA damage mostly by the indirect effect, which primarily involves the attack of OH radicals on the sugar and the base moieties. The ensuing chemical reactions are exceedingly complex [1], and different types of DNA damage have been documented. These types of damage include: (1) single- and double-strand breaks, (2) the formation of apurinic or apyrimidinic sites, and (3) chemically altered DNA bases. The single-strand breaks are usually removed by cellular enzyme repair systems, while double-strand breaks, depending on their nature, can be either lethal or mutagenic. Apurinic and apyrimidinic sites constitute an intact DNA phosphates diester - sugar backbone, but with a DNA base missing at a particular site. These lesions are known to have mutagenic characteristics if not successfully repaired by the normal complement of repair enzymes present in the cell.

Altered DNA bases are either, (1) repaired in an error-free or error-prone manner, (2) bypassed by DNA polymerases (if not repaired) with the correct or incorrect insertion of a base, or (3) DNA polymerase blocks. There are at least 29 different types of DNA-base alterations produced by ionizing radiation [2]. It is thus difficult to correlate specific DNA damage and biological end points. Overall, there is a similarity in the biological end points produced by ionizing radiation and by bulky PNA metabolic derivatives.

Both produce DNA damage which manifests itself ultimately in terms of these endpoints. Our long range goal is to elucidate what kind of DNA damage produces biologically deleterious effects, and which type of DNA damage does not.

In living cells, polynuclear aromatic compounds are metabolized by the P-450 enzyme system to a variety of oxygenated derivatives. Most of these are phenol derivatives which are readily excreted since their solubilities in the aqueous cellular environment are greater than those of the parent hydrophobic compounds. Unfortunately, some of the metabolic intermediates, notably the epoxide and diol epoxide derivatives, are highly reactive and bind chemically to biological macromolecules. The formation of covalent chemical addition products (or adducts) between the DNA bases and the PNA diol epoxide derivatives are known to give rise to DNA replication stops [3,4], and to mutations [5-7].

Because of the availability of isomeric polycyclic aromatic hydrocarbon (PAH) diol epoxide derivatives with dramatically different biological activities [8], these molecules provide ideal systems for studies of DNA damage-biological activity relationships.

b. Biological activities of polycyclic aromatic hydrocarbons.

We have been studying the diol epoxide derivatives of benzo(a)pyrene, chrysene, benz(a)anthracene, and benzo(a)phenanthrene, and their interactions with and binding to a variety of nucleic acids. In this report we summarize our recent work with the benzo(a)pyrene diol epoxide (BPDE) derivatives shown in Fig. 1. There are four stereoisomers of BPDE; we shall focus our attention on the two enantiomers of trans-7,8-dihydroxy-anti-9,10-epoxy-7,8,9,10,-tetrahydrobenzo(a)pyrene. The (+)-BPDE enantiomer (Fig. 1) is the most tumorigenic (in newborn mice) of all four stereoisomers of BPDE [8]; of particular interest from a point of view of biological activity-structure relationships,

FIGURE 1

Structures of the highly tumorigenic (+) enantiomer of BPDE, and the non-tumorigenic (-) enantiomer.

is the fact that the (-)-enantiomer ((-)-BPDE) is not tumorigenic, even though there is only a mirror-image relationships between the two isomers as is shown in Fig. 1. The mutagenic characteristics of these two isomers are also quite different, the (+)-enantiomer being significantly more mutagenic than (-)-BPDE in mammalian cell systems [5,8]. On the other hand, in bacterial systems, it is the (-)-enantiomer which is more mutagenic than the (+)-isomer [6,8]. The (+)-BPDE-DNA and (-)-BPDE-DNA adducts are processed quite differently by the enzymatic repair and/or replication machineries of prokaryotic and eukaryotic cells [5,6].

These differences in the biological activities of (+)-BPDE and (-)-BPDE, and of similar systems of isomeric PAH diol epoxide derivatives [8], may be related to the properties and structures of the PAH diol epoxide-DNA adducts [9]. Our ultimate aim is to distinguish between biologically deleterious and biologically benign PAH-DNA lesions. Of particular interest is to determine whether there is a general motif which distinguishes such mutagenic and non-mutagenic lesions from one another. Finally, can these considerations be extended to DNA base damage and mutagenicity induced by ionizing radiation?

Within the last year, we have developed new approaches towards an understanding of the basic interactions between the bulky hydrophobic PAH residues and neighboring DNA bases which provides valuable information on the properties of these mutagenic and carcinogenic PAH-DNA lesions.

B. OVERVIEW OF METHODS OF APPROACH.

Our approach involves synthesizing different isomeric BPDE - DNA adducts of known chemical composition, and the characterization of their physico-chemical and structural properties. Correlations between such in vitro model studies and in vivo BPDE-DNA adducts obtained from cells which had been treat-

ed with benzo[a]pyrene, is another very critical issue. Are the adducts the same, physically and chemically, obtained from cellular DNA and in laboratory model systems?

In a cellular environment, the concentration of BPDE moieties per DNA base varies from one in 20,000 (maximum), to one in 10^6 . In the DNA model systems on the other hand, BPDE-DNA adducts with levels of binding of one BPDE residue per 10 - 1000 DNA bases are usually prepared. In order to correlate results obtained from the cellular and laboratory model system experiments, highly sensitive spectroscopic and chemical techniques are required because of the low levels of binding *in vivo*. Thus, because of their relatively low sensitivities, high resolution NMR techniques are not suitable for this purpose. We have recently demonstrated that fluorescence techniques do have the required sensitivity [10,11]. We have also shown that the fluorescence characteristics of the pyrenyl residues at different BPDE-DNA binding sites are highly sensitive to their local microenvironment. Consequently, different types of BPDE-DNA adduct conformations can be distinguished by comparing the fluorescence characteristics with those obtained using other physico-chemical techniques [10].

Our overall approach, developed within the last 1-2 years is summarized in Fig. 2. We start from the lowest level of complexity which involves the chemical binding of one BPDE molecule to one nucleoside (deoxyguanosine, dG). In cellular DNA, as well as in native DNA in model systems, the dominant mode of covalent adduct formation involves a chemical bond between the C-10 atom of BPDE and the exocyclic amino group of dG [12]. A smaller fraction of BPDE molecules (less than 5% in the case of (+)-BPDE [12]) binds also to the exocyclic amino group of deoxyadenosine. The structures of both the BPDE-dG and BPDE-dA adducts are shown in Fig. 3 [12].

An additional adduct heterogeneity arises from the fact that epoxide-

**ADDUCTS SYNTHESIZED STARTING
WITH (+)-BPDE AND (-)-BPDE:**

1. cis- and trans-BPDE-dG

2. Single Strand Oligo

3. Double Strand Oligo

4. Synthetic and Cellular DNA:

FIGURE 2

Overview of methods of approach.

FIGURE 3
 Structures of major BPDE-dG adduct
 (left), and minor BPDE-dA adduct
 (right).

ring opening and binding to the N2 (exocyclic) amino group of dG (and dA) can proceed either by a trans- or by a cis-mechanism (Fig. 4).

Once the properties of the trans-BPDE-dG, or cis-BPDE-dG (or analogous BPDE-dA) adducts are characterized, these adducts can be site-specifically and base-sequence specifically, incorporated into a single-stranded, well-defined deoxyoligonucleotide (Fig. 2). We have devised efficient methods for synthesizing such defined BPDE-oligonucleotide adducts [13,14]. The single-stranded cis or trans-BPDE-oligonucleotide sequences can then be titrated with the complementary strands to form double-stranded, highly defined trans or cis mini-helix DNA-adducts. The physico-chemical characteristics of these BPDE-oligonucleotide adducts can then be compared with adducts obtained when BPDE binds to native DNA, or when benzo(a)pyrene is enzymatically activated to BPDE which then binds covalently to cellular DNA.

In this progress report, we summarize our recent work on the synthesis and characterization of chemically defined BPDE and BPDE-oligonucleotide adducts.

C. MECHANISMS OF INTERACTIONS BETWEEN THE PYRENYL AND BASE MOIETIES IN COVALENT BPDE-DEOXYNUCLEOSIDE ADDUCTS.

The physico-chemical interactions between the hydrophobic polycyclic aromatic residues and the neighboring DNA bases may interfere with the normal functioning of polymerases and other enzymes at or near the replication fork. Such hydrophobic interactions may represent polymerase stop sites and/or give rise to error-prone bypass replication. Therefore, it is important to characterize these interactions from a physico-chemical point of view.

Our initial focus here is on the basic unit of covalent BPDE-DNA adducts, namely the (+)-trans and (+)-cis-BPDE-dG adducts (Fig. 3), which

**STEREOCHEMISTRY OF ADDUCT FORMATION
FORMATION WITH GUANINES**

FIGURE 4

Stereochemistry of adduct formation.

were synthesized by the methods of Chang et al. [12], and subsequently subjected to detailed spectroscopic studies.

a. Optical absorption and circular dichroism spectra.

As compared to free BPDE in aqueous solution, the prominent absorption spectrum of the pyrenyl residue in BPDE-dG adducts shifts by at most 1 nm to the red (data not shown). This result suggests that there are at best minor pyrenyl-guanyl base-stacking interactions; these $\pi - \pi^*$ interactions are known to give rise to much larger (10 nm) red shifts [9].

The circular dichroism spectra are excitonic in nature, as reported earlier [15]; this shows that there are rather strong, adduct conformation-dependent interactions between the pyrenyl and guanyl (or guanosyl) residues. Such CD spectra (not shown), can therefore be used to characterize changes in the relative orientations of the two interacting moieties in covalent BPDE-dG complexes, and BPDE-oligonucleotide adducts.

b. Fluorescence characteristics.

The fluorescence and excitation spectra of trans-(+)-BPDE-dG adducts in aqueous methanol solutions are shown in Fig. 5. The fluorescence excitation spectra (Fig. 5, left-hand side) with maxima at 329 and 345 nm, resemble the absorption spectra in shape and positions of the maxima (not shown). The emission spectra are characterized by maxima at 379 and 399 nm. These features are characteristic of pyrenyl residues in aqueous solutions. There are several noteworthy features of the fluorescence characteristics of these adducts:

1. In water, the fluorescence yield is 100 times lower than that of the tetraol 7,8,9,10-tetrahydroxytetrahydrobenzo[a]pyrene (BPT). Thus, the

FIGURE 5

Fluorescence excitation (left) and emission spectra (right) of (+)-trans-BPDE-dG adducts in different H₂O/methanol solvent mixtures.

presence of the guanyl residue attached to the C10 position of the polycyclic aromatic molecule leads to a remarkable reduction in its fluorescence yield.

2. The fluorescence yield increases markedly, by a factor of about 8, as the methanol concentration is increased from 0 to 80% (Fig. 6). However, there are no concomitant changes in the shapes of the fluorescence spectra nor in the positions of the excitation or emission maxima as the solvent composition is varied.

3. The fluorescence decay profiles (measured at the National Synchrotron Light Source at Brookhaven, in collaboration with L. France and J.C. Sutherland) are non-exponential in shape (data not shown). Standard computer-analysis of the fluorescence decay profiles ($F(t)$) shows that in all cases, the fluorescence decay is adequately described by a two-component, double-exponential model:

$$F(t) = A_1 \exp\{-t/\tau_1\} + A_2 \exp\{-t/\tau_2\} \quad [1]$$

where τ_1 and τ_2 are the characteristic decay times of the two components whose amplitudes are A_1 and A_2 . These latter two quantities are proportional to the relative abundances of the two species which decay with fluorescence lifetimes τ_1 and τ_2 .

Some typical fluorescence decay parameters are summarized in Table 1 for a (+)-trans-BPDE-dG and a (+)-cis-BPDE-dG adduct in pure water and in a 20% H₂O/80% methanol mixture. In the case of the (+)-trans-adduct and in 100% water, there are two fluorescence lifetimes of 0.86 and 2.0 ns, with relative amplitudes of 37% and 63%, respectively. The fluorescence quantum yield (Q) of the (+)-trans-BPDE-dG adduct relative to that of the tetraol BPT, can be estimated from these parameters according to the equation [16]:

$$Q = \frac{A_1 \tau_1 + A_2 \tau_2}{\tau(\text{BPT})} \quad [2]$$

FIGURE 6

Fluorescence yield (relative to BPT tetraols) of (+)-*trans*-BPDE-dG adducts as a function of % methanol in water.

TABLE I.
FLUORESCENCE DECAY TIMES (τ_i)
AND AMPLITUDES (A_i) in MeOH/H₂O
(+)-trans-BPDE-dG

	$\tau_1(A_1)$	$\tau_2(A_2)$
100% H ₂ O	0.86 ns (37%)	2.0 ns (63%)
20% H ₂ O	1.3 ns (6%)	15 ns (94%)

(+)-cis-BPDE-dG

100% H ₂ O	0.15 ns (95%)	2.7 ns (5%)
20% H ₂ O	0.27 ns (85%)	5.8 ns (15%)

where $\tau(\text{BPT}) = 200 \text{ ns}$, is the fluorescence decay time of the tetraol BPT. Utilizing the data in Table 1, a relative quantum yield of 0.008 is calculated from the decay profile parameters which is quite close to the relative fluorescence quantum yield of 0.01 measured directly from the relative steady-state fluorescence yields of BPT and of the (+)-*trans*-BPDE-dG adducts (Fig. 6).

In 80% methanol solution, the fluorescence decay times, especially of the longer component of the (+)-*trans*-BPDE-dG adduct, are markedly longer than in 100% water; the amplitude of the shorter component is significantly lower at the higher methanol concentration (Table 1).

In the case of the (+)-*cis*-BPDE-dG adduct, analogous behavior is observed, but the effects of methanol on the fluorescence decay times of the second component and on the amplitudes are much less pronounced than in the case of the *trans*-adduct (Table 1). These results indicate that the fluorescence characteristics are strongly dependent on the relative conformations of the guanyl and pyrenyl moieties which are known to be different from one another in *trans* and *cis* adducts [12].

Taken together, the fluorescence characteristics of the BPDE-dG adducts defined in 1. - 3. above, are consistent with an electron-transfer exciplex model originally formulated for other fluorescence-emitting electron transfer donor-emitter collision complexes in solvent mixtures of varying polarities [17]. We have previously observed such behavior in the case of the aromatic carcinogen 2-aminofluorene bound covalently to the C8 position of deoxyguanosine (previous progress reports).

In Weller's exciplex model, a polycyclic aromatic molecule collides with a fluorescence quencher (typically an aromatic amine derivative) and forms a transient complex $C^\pm$ with a considerable charge transfer component. In

highly polar solvents, the intermediate charge transfer complex dissociates to radical ions which ultimately recombine to form non-fluorescent triplet states or excited ground-state species. In solvents of low polarity, on the other hand, the formation of charged species is less favored and the equilibrium favors the initial, fluorescence-emitting singlet excited state of the polycyclic aromatic hydrocarbon. Thus, solvent mixtures of increasing polarity are associated with lower fluorescence quantum yields, as is indeed observed in the case of the (+)-trans-BPDE-dG adducts (Fig. 6).

Weller's exciplex model adapted to covalent BPDE-dG adducts may be summarized as follows:

In this model, the formation of the charge-transfer complex is favored the higher the polarity of the environment, for example, in 100% water. The individual charged species are stabilized by interaction with water molecules which may lower the energy of the complex below that of the singlet excited state of the pyrenyl residue. Consequently, the greater the probability of formation of the charge-transfer exciplex, the lower the fluorescence yield of the system, assuming that the charge-transfer states themselves do not contribute to the fluorescence. The fluorescence spectra shown in Fig. 5 obtained in different H₂O/MeOH mixtures are of the molecular type, i.e., they are due to the pyrenyl residue alone. Red-shifted, broad emission spectra as are often observed in the case of exciplexes, are not evident in the case of

the BPDE-dG adducts. Therefore, our assumption that the charge transfer states on the right-hand side of scheme 3 are non-fluorescent, is justified.

The presence of two lifetime components, with the amplitude of the faster one decreasing with decreasing solvent polarity, suggests that there are two (or perhaps more than two) fluorescence-emitting species. The faster decaying component whose amplitude increases with increasing solvent concentration, arises most likely from a mechanism of the type described by Eq. 3. The second component, whose amplitude decreases with increasing solvent polarity, may be due to species with a different adduct micro-conformation, or due to fluorescent species which are quenched by mechanisms other than those involving electron transfer. These issues have not yet been clarified.

In scheme 3, we have indicated that electron transfer occurs from the guanyl moiety to the pyrenyl ring. This assumption is justified by two additional characteristics of BPDE-dG complexes which will now be discussed.

4. The pH dependence of fluorescence yields of (+)-trans-BPDE-dG adducts.

It is well known in the field of nucleic acid chemistry that at pH values below ~ 3, the N7 position becomes protonated and the guanyl residue becomes positively charged. Electron transfer from the guanyl residue to the pyrene residue should be hindered by this positive charge. At pH values above 10, the proton at the N1 position is removed, and the guanyl residue assumes an overall negative charge; electron transfer from the guanyl residue to the pyrenyl residue should be enhanced in this case. These considerations suggest that, if the fluorescence is indeed quenched by electron transfer from dG to the BPDE residue (scheme 3), then the fluorescence yield should be enhanced at pH values below 3, and be decreased at pH values above 10. These predic-

The intensity of fluorescence of
Trans-(+)-BPDE-dG as a function of pH

FIGURE 7

Relative fluorescence yield of (+)-trans-BPDE-dG adducts in aqueous solutions as a function of pH.

tions are indeed borne out by the experimental results shown in Fig. 7. At pH 1.0, the fluorescence yield of (+)-trans-BPDE-dG adducts in aqueous solution is about 10 times higher than in the pH region 3 - 9. On the other hand, at pH 12, the yield is ten times lower than at neutral pH. Thus, the fluorescence yield decreases by a factor of 100 as the pH is increased from 1.0 to 12.0. This behavior is consistent with scheme 3.

5. Laser-induced transient kinetic absorption spectra of (+)-trans-BPDE-dG adducts.

A direct confirmation of scheme 3 would be the demonstration that the pyrenyl residue is indeed negatively charged at least for a short time period after photoexcitation, corresponding to the lifetime of the putative $\text{BPDE}^- \text{-dG}^+$ charge transfer complex on nanosecond time scales (Table 1). Experiments along these lines were performed by our collaborator P. Pluzhnikov at the Radiation Laboratory at Notre Dame University. A picosecond pulse Nd:Yag laser operating at 355 nm excited the BPDE-dG adducts at 355 nm, and the transient absorption spectra of the short-lived intermediate species were measured on nanosecond time scales utilizing a white continuum and an optical multichannel analyzer system. A typical transient absorption spectrum, measured ~ 100 ps after the excitation flash, is shown in Fig. 8. This spectrum, obtained in 20% H_2O /80 % MeOH mixtures, decays with a mean decay time of about 3.8 ns, which is somewhat slower although in the same time range as the fast fluorescence decay component observed in this same solvent mixture (Table 1).

The transient absorption spectrum exhibits a maximum at 490 nm. As Weller and his co-workers have shown [18], such a transient spectrum with a maximum at 490 nm, is characteristic of negatively charged pyrene (Py^-). We therefore conclude that the fluorescence quenching mechanism of pyrenyl resi-

FIGURE 8

Transient absorption spectrum of (+)-trans-BPDE-dG adducts in aqueous solution (100 ps after excitation with a picosecond 355 nm laser flash). (Data of P. Pluzhnikov, U. Notre Dame).

dues in covalent BPDE-dG adducts indeed involves an electron transfer from the guanyl to the pyrenyl residue, as suggested in scheme 3 above.

This fluorescence quenching mechanism is strongly dependent on the conformations and relative orientations of the pyrenyl residue and the dG moiety, as well as on the local polarity of the immediate solvent environment. These characteristics are of importance for characterizing the conformations and properties of more complex BPDE-DNA adducts both *in vitro* and *in vivo*. A few such applications and some preliminary data are discussed in the next section.

D. FLUORESCENCE TECHNIQUES FOR DISTINGUISHING DNA ADDUCTS DERIVED FROM HIGHLY TUMORIGENIC AND NON-TUMORIGENIC STEREOISOMERS OF BPDE.

a. Site I and Site II adduct conformations: correlations with *cis* and *trans* BPDE-dG addition products.

As mentioned in the Introduction, the four stereoisomers of BPDE are characterized by remarkable differences in their biological activities. We have shown previously [9] that the structures of the adducts formed when BPDE binds covalently to native, double-stranded DNA are strikingly different for the tumorigenic (+)-BPDE and non-tumorigenic (-)-BPDE isomers (Fig. 1). Two types of binding sites, termed "site I" and "site II" have been recognized. The (+)-BPDE isomer gives rise to adducts which are predominantly of type II, while (-)-BPDE gives rise predominantly to site I adducts upon binding covalently to native DNA. The characteristics of these two types of binding sites are summarized in Table 2.

The major, spectroscopically observable differences involve variations in the red-shifts of the absorption and fluorescence spectra [10,19]. The maximum in the absorption or fluorescence excitation spectrum of BPT in aque-

TABLE 2

**DISTINGUISHING FEATURES OF
CONFORMATIONS OF COVALENT DNA
ADDUCTS DERIVED FROM (+)-BPDE AND
(-)-BPDE.**

**■ ADDUCT CONFORMATIONS ARE VERY
DIFFERENT:**

- (-)-BPDE forms predominantly SITE I -
type adducts
- (+)-BPDE forms predominantly SITE II -
type adducts

**■ CHARACTERISTICS OF SITE I AND
SITE II ADDUCTS:
(N2 of deoxyguanosine)**

<u>SITE I</u>	<u>SITE II</u>
Absorption Spectra: 10 nm red shift	Only a 2-3 nm red shift
Pyrene plane appx. perpendicular to helix axis	Plane appx. parallel to helix axis
Hydrophobic binding site	Exposed to H ₂ O environment
Pyrene-base stacking interactions	Minimal stacking interactions

ous buffer solution, in the absence of DNA, occurs at 343 nm. In site I adducts, this maximum is red-shifted by about 10-11 nm, and is observed at 353-354 nm; in site II adducts the red shift is only 2-3 nm, and the absorption maxima are located at 345-346 nm. These differences are attributed to extensive $\pi - \pi^*$ pyrenyl - DNA base-stacking interactions due to parallel, or near-parallel (within 30°) relative orientations of the planar ring systems in site I adducts. In site II adducts on the other hand, the red-shift is much smaller because the pyrenyl residue is located in a solvent-exposed DNA binding site, most likely in the minor groove of a locally altered B-DNA structure. Computer modeling and energy-minimization calculations by S. Broyde (NYU) and B. Hingerty (Oak Ridge National Laboratory) have shown that such site I and site II structures are feasible; typical examples derived from their work are shown in Fig. 9. The local bends at the binding sites are particularly pronounced in the case of the site II structure, and are in accord with experimental observations [9,20].

On the basis of studies of conformations of adducts derived from the binding of nearly 20 different PAH diol epoxide isomers to native, double-stranded DNA, we have noted a correlation between adduct conformation and biological (tumorigenic) activities of these isomers [9]. The most highly active isomers give rise to site II adducts, while their less active, or inactive isomers, give rise to site I - type adduct conformations. The fact that there are two types of BPDE-dG and BPDE-DNA adducts, *cis* and *trans*, led us to hypothesize that the formation of *cis*- and *trans*-BPDE-DNA adducts is related to the observation of site I and site II adduct species. This hypothesis is in full accord with the experimental evidence we collected within the last year, and which is described below.

FIGURE 9

Computer models of site II (left) and site I (right) BPDE-(dG-dC)₉ (Courtesy of S. Broyde and B. Hingerty).

b. Preparation of site-specific BPDE-deoxyoligonucleotides and correlations of structure with biological activities.

In order to investigate the basis of this apparent correlation from a chemical and structural point of view, we initiated studies with site-specifically modified oligonucleotides (see scheme, Fig. 2). This approach is more specifically summarized in Fig. 10. We first synthesized single-stranded deoxyoligonucleotides of well defined base sequences containing a single dG residue; since (+)-BPDE molecules react preferentially with dG (> 95%, [12]), this approach, utilizing a single dG residue, limits the number of adducts which are obtained. The single-stranded oligonucleotides were then allowed to react with BPDE in aqueous solutions. The different reaction products, consisting mostly of (+)-*cis* and (+)-*trans* BPDE-N2-dG addition products, were separated from one another, and purified by HPLC. A typical HPLC elution profile is shown in Fig. 11. The yields with the sequence d(ATATGTATA) are particularly high under the experimental conditions employed which are described in greater detail elsewhere [13].

The properties of each of the products isolated, either the (+)-*cis* or the (+)-*trans* d(ATAT[G^{*}-BPDE]TATA) adducts, were characterized by biochemical and other techniques to ascertain the stereospecificity of each adduct [13]. Subsequently, the complementary strand d(TATACATAT) was added stoichiometrically in order to form the double-stranded adducts and to investigate their physico-chemical characteristics.

c. Characteristics of single-stranded BPDE-oligonucleotide adducts.

The characteristics of the *cis* and *trans* single-stranded (+)-d(ATAT[G^{*}-BPDE]TATA) adducts have been discussed in detail elsewhere [13]. The absorption spectra of these single-stranded adducts suggest that $\pi - \pi^*$ carcinogen-

**SCHEMATIC DIAGRAM OF THE STRUCTURE
OF A TYPICAL OLIGODEOXYRIBONUCLEOTIDE STUDIED.**
A: SINGLE STRAND
B: SINGLE STRAND WITH COMPLEMENT

(+)BaPDE-d(ATATGTATA)
~34% OF DNA STRANDS REACTED
~20% OF (+)BaPDE ADDED REACTED
Ratio of trans:cis N2 guanine adducts = 7:1

FIGURE 11

HPLC elution profiles of (+)-BPDE-d(ATATGTATA) reaction mixtures [13].

base stacking interactions are considerable even in these single stranded adducts. In fact, the fluorescence characteristics suggest that the pyrenyl residues bound covalently to the N2 exocyclic amino group of dG in the (+)-d(ATAT[G^{*}-BPDE]TATA) adducts are not as completely exposed to the aqueous solvent environment as site II-type BPDE-DNA adducts. This is shown in Fig. 12, in which the fluorescence yield of (+)-trans-d(ATAT[G^{*}-BPDE]TATA) adducts is plotted as a function of the H₂O/MeOH solvent composition. First, in 100% water, the fluorescence yield relative to that of the tetraols BPT is 0.21, which is some 20 times higher than that of the monomeric (+)-trans-BPDE-dG adduct in 100% aqueous solution (Fig. 6). As the methanol concentration is increased to 80 %, the fluorescence yield increases by another factor of 7 (Fig. 12). A 2-3 nm blue shift in the absorption maximum of the BPDE-oligonucleotide adducts accompanies this increase in fluorescence yield; also, a similar blue-shift in the absorption spectrum is observed when the aqueous (+)-BPDE-oligonucleotide solutions are heated to about 80° C (data not shown). Both of these observations suggest that the pyrenyl-DNA base stacking interactions in (+)-d(ATAT[G^{*}-BPDE]TATA) adducts are disrupted either when the methanol concentration is increased or when the temperature is raised.

Because of the low thermodynamic stabilities of the double-stranded 9-mer (+)-d(ATAT[G^{*}-BPDE]TATA)•d(TATACATAT) (see below, and reference 14), another sequence, d(CACATGTACAC) containing additional deoxycytosines, were synthesized. The additional dC's in the (+)-BPDE-modified single strands offer opportunities for dG-dC base pairing in the double-stranded forms, thus stabilizing the BPDE-modified double helices. Also, the chain length was increased to 11 bases (11-mer). The (+)-BPDE-modified d(CACATGTACAC) sequences had absorption and fluorescence characteristics similar to those of the d(ATATGTATA) 9-mer sequences described here [14].

FIGURE 12

Fluorescence yield of single-stranded d(ATAT[G*-BPDE]TATA) adduct as a function of methanol concentration in aqueous solution. Vertical scale is expressed relative to the fluorescence yield of the tetraols BPT, and can thus be compared directly to the yield values in Fig. 6.

FLUORESCENCE INTENSITY

FIGURE 13

Fluorescence excitation (left) and emission (right) spectra of (+)-cis adducts:

(A) (+)-cis-d(CACAT[G*-BPDE]TACAC), single strands.

(B) Double-stranded form: same as in A, but titrated with unmodified complementary strand.

FIGURE 14

Fluorescence excitation (left) and emission (right) spectra of (+)-trans-adducts:

(A) (+)-trans-d(CACAT[G^{*}-BPDE]TACAC), single stranded form.

(B) Double-stranded form: same as in A, but titrated with unmodified complementary strand.

The fluorescence excitation and emission spectra of the (+)-*cis*- and (+)-*trans*-d(CACAT[G^{*}-BPDE]TACAC) adducts are shown in Figs. 13A and 14A, respectively. The excitation maxima occur in both cases at 335 and 350 - 351 nm, while the maxima in the emission spectra occur at 383 - 385 and 401 - 405 nm. These spectra are red-shifted by about 6 - 7 nm with respect to the analogous maxima observed in aqueous solutions of tetraols in the absence of DNA [21].

d. Characteristics of double-stranded BPDE-oligonucleotide adducts.

When the complementary 11-mer strands d(GTGTACATGTG) are stoichiometrically added to the (+)-d(CACAT[G^{*}-BPDE]TACAC) strands, stable duplexes are formed at or near room temperature. The melting points of the unmodified (CACATGTACAC)•d(GTGTACATGTG) duplexes are 39° C. The BPDE-modified oligonucleotide complexes (+)-*trans*-d(CACAT[G^{*}-BPDE]TACAC)•d(GTGTACATGTG) and (+)-*cis*-d(CACAT[G^{*}-BPDE]TACAC)•d(GTGTACATGTG) are characterized by melting points of 27° and 41° degrees, respectively. Thus, the (+)-*trans*-adducts are destabilized, while the (+)-*cis*-adducts are stabilized by the presence of the covalently bound BPDE moieties at the N2 position of deoxyguanosine in the oligonucleotides. Apparently, the additional stabilization in the *cis* adducts may be due to the effects of carcinogen-base stacking.

The fluorescence excitation and emission profiles of the double-stranded (+)-*cis*- and (+)-*trans*-11-mer oligonucleotides are very interesting in that they exhibit quite different characteristics (Figs. 13B and 14B). Upon adding the complementary strand, there is a further 1-2 nm red shift in the excitation and emission spectra of the (+)-*cis*-adducts; this is evident from a comparison of the single-stranded adducts (Fig. 13A) and the double-stranded (Fig. 13B) adduct forms.

The behavior of the (+)-*trans*-adducts, on the other hand, is strikingly

different (Fig. 14). Upon addition of the complementary strand to the (+)-trans-d(CACT[G^{*}-BPDE]TACAC) adduct, there is a blue shift in the excitation and emission spectra by 4-5 nm! Apparently, the base-stacking interactions in the single-stranded adducts are considerably diminished upon double-strand formation. In the double-stranded form, the maxima in the excitation spectrum occur at 331 and 346 nm (Fig. 14B, solid line), in contrast to the analogous spectrum of the (+)-cis-adduct whose maxima are located at 339 and 353 nm (Fig. 13B, or Fig. 14B, dotted line). Changes analogous to those in the fluorescence excitation spectra are also observed in the absorption spectra when the complementary strands are added to the single-stranded (+)-trans- and (+)-cis-11-mers.

e. Identification of conformational characteristics of BPDE-DNA lesions and tumorigenic activities.

The striking resemblances of the fluorescence excitation and absorption spectra of the trans- and cis-BPDE-oligonucleotide adducts to those of site I and site II BPDE-native DNA adducts (Table 2), suggest the following [14]:

i. Site I - type adducts can be identified with cis-addition products of the BPDE stereoisomers to the exocyclic amino group of deoxyguanosine in native DNA.

ii. Site II - type adducts can be identified with trans-addition products of BPDE stereoisomers to the exocyclic amino group of deoxyguanosine in DNA.

Coupled with our previous hypothesis that tumorigenic activity and relatively high mutagenic activity of BPDE and similar PAH diol epoxide stereoisomers are associated with the formation of site II - type of adducts [9], the external conformation of the pyrenyl residues in site II - DNA adducts may

be associated with physico-chemical characteristics which lead to mutations either due to faulty enzymatic DNA repair, or faulty DNA replication.

Finally, we discuss differences in biological activities and DNA adduct structures arising from the binding of the tumorigenic (+)-BPDE and non-tumorigenic (-)-BPDE (Fig. 1). In native DNA, the binding of the (+)-enantiomer gives rise to 94% (+)-trans-BPDE-N2-dG adducts, and only 1% (+)-cis adducts, and 5% deoxyadenosine (dA) adducts; (-)-BPDE, on the other hand, gives rise to 63% (-)-trans-BPDE-N2-dG adducts and 22% of the analogous (-)-cis-adducts, and 15% of (-)-trans and (-)-cis-dA adducts [12]. Thus, in the case of (+)-BPDE, site II trans adducts dominate, as is found experimentally [22]. Our model predicts that (-)-BPDE-native DNA adducts should be much more heterogeneous and should consist of mixtures of site I and site II adduct conformations; this prediction is also borne out by the experiments [22].

We conclude that one of the factors involved in the high tumorigenicity of (+)-BPDE is its preferred formation of site II (+)-trans-adducts. In the case of (-)-BPDE, the incidence of (-)-trans-adducts is lower, but not sufficiently lower to account for the lack of tumorigenicity of this isomer. It is likely that the trans-dG adducts derived from (+)-BPDE and (-)-BPDE differ in some important details which further accounts for their differences in biological activities.

E. CONCLUSIONS AND FUTURE OUTLOOK.

Our recent findings open up new directions of research in structural chemistry, theoretical modeling, and molecular biology. High resolution NMR and X-ray crystallographic studies on defined trans- and cis-BPDE and other PAH diol epoxide-oligonucleotide adducts should now become a possibility, since we have shown in our laboratory that several mg of pure adducts can be

synthesized. NMR experiments are presently underway. Theoretical computer modeling studies, in collaboration with our colleague S. Broyde at New York University, are also in progress. Exciting new developments in molecular biology involving site-specific BPDE-oligonucleotides are also possible. For example, site-directed mutagenesis studies [23] using highly defined *cis*- and *trans*-PAH diol epoxide-oligonucleotide vectors should be performed in order to test the hypothesis concerning the differences in the mutagenic activities of these two types of DNA adduct lesions. Other types of investigations, for example whether these two types of adducts are bypassed during replication or act as replication stops, or whether one or the other lesion is more readily repaired *in vivo*, should also be performed.

REFERENCES FOR PART III.

- [1]. C. von Sonntag, *The Chemical Basis of Radiation Biology*, Taylor & Francis, London (1987).
- [2]. Téoule, R., *Int. J. Rad. Biol.* 51, 573 (1987).
- [3]. J. Sahn, E. Turkington, D. LaPointe and B. Strauss, *Biochemistry* 28, 2836 (1989).
- [4]. M. Cordeiro-Stone, J.C. Boyer, B.A. Smith and W.K. Kaufmann, *Carcinogenesis* 7, 1775 (1986).
- [5]. P. Brookes and M.R. Osborne, *Carcinogenesis* 3, 1223 (1982).
- [6]. C.W. Stevens, N. Bouck, J.A. Burgess and W.E. Fahl, *Mutat. Res.* 152, 5 (1985).
- [7]. J.-L. Yang, V.M. Maher and J.J. McCormick, *Proc. Natl. Acad. Sci.* 84, 3787 (1987).
- [8]. A.H. Conney, *Cancer Res.* 42, 4875 (1982).
- [9]. N.E. Geacintov, in: *Polycyclic aromatic Hydrocarbons Carcinogenesis: Structure-Activity Relationships*. S.K. Yang and B.D. Silverman, Eds., CRC Press, Boca Raton, FL, Vol. II, pp. 181-206.
- [10]. S.K. Kim, N.E. Geacintov, H.C. Brenner and R.G. Harvey, *Carcinogenesis* 10, 1333 (1989).

- [11]. R. Jankowiak, P.-Q. Lu, G.J. Small and N.E. Geacintov, *Chem. Res. Toxicol.* 3, 39 (1990).
- [12]. S.C. Cheng, B.D. Hilton, J.M. Roman and A. Dipple, *Chem. Res. Toxicol.* 2, 334 (1988).
- [13]. M. Cosman, V. Ibanez, N.E. Geacintov and R.G. Harvey, *Carcinogenesis* 00, 000 (1990), in press.
- [14]. N.E. Geacintov, M. Cosman, V. Ibanez, S.S. Birke and C.E. Swenberg, in: *Molecular Base Specificity in Nucleic Acid-Drug Interactions*. B. Pullman and J. Jortner, Eds., Kluwer Academic Publ., Dordrecht, 1990, in press.
- [15]. K. Nakanishi, H. Kasai, H. Cho, R.G. Harvey, A.M. Jeffrey, K.W. Jennette, and I.B. Weinstein, *J. Am. Chem. Soc.* 99, 258 (1977).
- [16]. N.E. Geacintov, *Photochem. Photobiol.* 45, 547 (1987).
- [17]. H. Beens and A. Weller, in: *Organic Molecular Photophysics*, J.B. Birks, Ed., John Wiley & Sons, London, Vol. II, pp. 159-215.
- [18]. H. Schomburg, H. Staerk and A. Weller, *Chem. Phys. Lett.* 21, 433, and 22, 1 (1973).
- [19]. S.K. Kim, H.C. Brenner, B.J. Soh and N.E. Geacintov, *Photochem. Photobiol.* 50, 327 (1989).
- [20]. A. Gräslund and B. Jernström, *Quart. Rev. Biophys.* 22, 1 (1989).
- [21]. V. Ibanez, N.E. Geacintov, A.G. Gagliano, S. Brandimarte and R.G. Harvey, *J. Am. Chem. Soc.* 102, 5661 (1980).
- [22]. D. Zinger, N.E. Geacintov and R.G. Harvey, *Biophys. Chem.* 27, 131 (1987).
- [23]. A.K. Basu and J.M. Essigman, *Chem. Res. Toxicol.* 1, 1 (1988).

Publications: January 1, 1989 -August 31, 1990, Acknowledging DOE Support.

1. Photoemission From Adenine Crystal: Solvation of a Pre-ionizing State. M. Zielinski, M. Pope, N. Wang, C. Horvath, and N.E. Geacintov, Chem. Phys. Lett. 165, 297-301 (1990).
2. Electrolysis at an Anthracene Crystal/Aqueous NO_3^- Solution Interface. M. Pope, B. Mao, J. Steigman, and N.E. Geacintov, J. Phys. Chem. 94, 2506-7 (1990)
3. Linear Dichroism Characteristics of Ethidium- and Proflavine-Supercoiled DNA Complexes. C.E. Swenberg, S.E. Carberry, and N.E. Geacintov, Biopolymers 29, 1735-1744 (1990).
4. Laser Spectroscopic Studies of DNA Adduct Structure Types from Enanti-
omeric Diol Epoxides of Benzo[a]pyrene. R. Jankowiak, P.-Q. Lu, G.J. Small, and N.E. Geacintov, Chem. Res. Toxicol. 3, 39-46 (1990).
5. Fluorescence Spectroscopy of Benzo[a]pyrene Diol Epoxide-DNA Adducts. Conformation-Specific Emission Spectra. S.-K. Kim, H.C. Brenner, B.J. Soh, and N.E. Geacintov, Photochem. Photobiol. 50, 327-337 (1989).
6. The Triplet State as a Probe of Dynamics and Structure in Biological Macromolecules. N.E. Geacintov and H.C. Brenner, Photochem. Photobiol. 50, 841-858 (1989).
7. Linear Dichroism Properties and Orientations of Different Ultraviolet Transition Moments of Benzo[a]pyrene Bound Noncovalently and Covalently to DNA. C.J. Roche, N.E. Geacintov, V. Ibanez, and R.G. Harvey, Biophys. Chem. 33, 277-288 (1989).
8. Reactions of Stereoisomeric Non-Bay-Region Benz[a]anthracene Diol Epoxides with DNA and Conformations of Non-Covalent Complexes and Covalent Adducts. S.E. Carberry, N.E. Geacintov, and R.G. Harvey, Carcinogenesis 10, 97-103 (1989).
9. Identification of Conformationally Different Binding Sites in Benzo[a]pyrene Diol Epoxide-DNA Adducts by Low Temperature Fluorescence Spectroscopy. S.-K. Kim, N.E. Geacintov, H.C. Brenner, and R.G. Harvey, Carcinogenesis 10, 1333-1335 (1989).
10. Interactions of the Dimethyldiazaperopyrenium Dication with Nucleic Acids. 2. Binding to Double-Stranded Polynucleotides. A. Slama-Schwok, M. Rougee, V. Ibanez, N.E. Geacintov, T. Montenay-Garestier, J.-M. Lehn, and C. Helene, Biochemistry 28, 3234-3242 (1989).
11. Fluorescence Spectral Characteristics and Fluorescence Decay Profiles of Covalent Polycyclic Aromatic Carcinogen-DNA Adducts. S.-K. Kim, N.E. Geacintov, D. Zinger, and J.C. Sutherland. in Synchrotron Radiation in Structural Biology. Basic Life Sciences, Vol. 51, (R.M. Sweet & A.D. Woodhead, eds.) Plenum Press, New York, pp. 187-205 (1989).

12. Excited State Properties of the N-(Deoxyguanosin-8-yl)-2-Aminofluorene Adducts. L.P.A. van Houte, R. van Grondelle, J. Retel, J.G. Westra, D. Zinger, J.C. Sutherland, S.K. Kim, and N.E. Geacintov, Photochem. Photobiol. 49, 387-394 (1989).

Other Publications

1. Differences in Conformations of Covalent Adducts Derived from the Binding of 5- and 6-Methylchrysene Diol Epoxide Stereoisomers to DNA. N.E. Geacintov, M.-S. Lee, V. Ibanez, S. Amin, and S.S. Hecht, Carcinogenesis 11, 985-989 (1990).
2. The Shape of the Pump-Probe Fluorescence Induction Curves in Chloroplasts is Determined by the Duration of the Pump Light Flash. L.L. France, N.E. Geacintov, and J. Breton, in: Current Research in Photosynthesis, Vol.I, (M. Baltscheffsky, ed.) pp. 467-470 (1990).
3. Linear Dichroism and Orientation of Chromophores in Nostoc sp. Phycobilisomes and Their Subunits. L.J. Juszczak, B.A. Zilinskas, N.E. Geacintov, and J. Breton, in: Current Research in Photosynthesis, Vol.II, (M. Baltscheffsky, ed.) pp. 125-128 (1990).

LIST OF PERSONNEL OF THE RADIATION AND SOLID STATE LABORATORY

SENIOR SCIENTISTS

Nicholas E. Geacintov, Director and Professor of Chemistry
Martin Pope, co-Director and Professor of Chemistry,
emeritus
Benson R. Sundheim, Professor of Chemistry
Henry C. Brenner, Associate Professor of Chemistry
Suse Broyde, Professor of Biology

RESEARCH SCIENTISTS

Victor Ibanez
Seog-Kyu Kim
Yuri Mnyukh
Tong-Ming Liu
Leonid Margulis

ADMINISTRATIVE AND TECHNICAL STAFF

Lillian Bloom
Therese Powers

GRADUATE STUDENTS

Anna Alfano
Luisa Balasta
Sheryl Birke
Monique Cosman
Laura Juszczak
Bing Mao
Shih-Chang Sheu
Suresh Singh
Deming Song
Ronnie Xu
Nai-Qi Ya
Rushen Zhao

UNDERGRADUATE STUDENTS

Sandra Chen
Willie Lee
Lorenzo Pecora
Fai Zen

VISITING SCIENTISTS

Jacques Breton, Paris, France
Vladimir A. Kuzmin, Moscow, USSR
Sergei Smirnov, Moscow, USSR

- END -

DATE FILMED

11 / 16 / 90