POLYCYCLIC-AROMATIC-HYDROCARBON-INDUCED ALTERATIONS IN THE PHYSIO-CHEMICAL CHARACTERISTICS OF ESCHERICHIA COLI DEOXYRIBONUCLEIC ACID

APPROVED:

Graduate Committee:

[Signatures of committee members]

Dean of the Department of Biology

Dean of the Graduate School
POLYCYCLIC-AROMATIC-HYDROCARBON-INDUCED ALTERATIONS IN THE PHYSIO-CHEMICAL CHARACTERISTICS OF ESCHERICHIA COLI DEOXYRIBONUCLEIC ACID

THESIS

Presented to the Graduate Council of the North Texas State University in Partial Fulfillment of the Requirements

For the Degree of

Doctor of Philosophy

By

J. Frederick Chapel, B. A., M. A.

Denton, Texas

August, 1969
Copyright by
James Frederick Chapel

1969
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF ILLUSTRATIONS</td>
<td>vi</td>
</tr>
<tr>
<td><strong>Chapter</strong></td>
<td></td>
</tr>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II. MATERIALS AND METHODS</td>
<td>9</td>
</tr>
<tr>
<td>Hydrocarbon Purification</td>
<td></td>
</tr>
<tr>
<td>Ethidium Bromide Purification</td>
<td></td>
</tr>
<tr>
<td>In Vivo Hydrocarbon-DNA-Reaction System</td>
<td></td>
</tr>
<tr>
<td>In Vivo Ethidium Bromide-DNA-Reaction System</td>
<td></td>
</tr>
<tr>
<td>Growth Assays</td>
<td></td>
</tr>
<tr>
<td>Preparation of DNA</td>
<td></td>
</tr>
<tr>
<td>Purification of Commercial DNA</td>
<td></td>
</tr>
<tr>
<td>In Vitro Hydrocarbon-DNA-Reaction System</td>
<td></td>
</tr>
<tr>
<td>In Vitro Ethidium Bromide-DNA-Reaction System</td>
<td></td>
</tr>
<tr>
<td>Measurement of Absorption Spectra</td>
<td></td>
</tr>
<tr>
<td>Differential Spectra</td>
<td></td>
</tr>
<tr>
<td>Melting-Temperature (T_m) Measurements</td>
<td></td>
</tr>
<tr>
<td>Spectral Analysis of Native DNA (ANDS - 1)</td>
<td></td>
</tr>
<tr>
<td>Viscosity Measurements</td>
<td></td>
</tr>
<tr>
<td>III. RESULTS</td>
<td>25</td>
</tr>
<tr>
<td>IV. DISCUSSION</td>
<td>32</td>
</tr>
<tr>
<td>APPENDIX I</td>
<td>40</td>
</tr>
<tr>
<td>APPENDIX II</td>
<td>49</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>53</td>
</tr>
<tr>
<td>Table</td>
<td>Page</td>
</tr>
<tr>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>I. Ultraviolet Absorption Peaks of Aromatic Hydrocarbons</td>
<td>26</td>
</tr>
<tr>
<td>II. Differential-Ultraviolet-Absorption Peaks for Ethidium Bromide and Aromatic Hydrocarbons</td>
<td>28</td>
</tr>
<tr>
<td>III. $T_m$ and $&lt;s&gt;$ Values for In Vitro Aromatic-Hydrocarbon and Ethidium Bromide-Reacted DNAs in 0.02 M NaCl-Tris</td>
<td>29</td>
</tr>
<tr>
<td>IV. The In Vivo Effects of Aromatic Hydrocarbons and Ethidium Bromide on the Characteristics of Escherichia coli DNA</td>
<td>30</td>
</tr>
</tbody>
</table>
LIST OF ILLUSTRATIONS

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. A Generalized Rotating Cylinder Viscometer.</td>
<td>20</td>
</tr>
<tr>
<td>2. The Effect on the Growth of <em>Escherichia coli</em> B of Aromatic Hydrocarbons and Ethidium Bromides</td>
<td>50</td>
</tr>
<tr>
<td>3. The Absorption Spectra of <em>in vitro</em> Polycyclic-Hydrocarbon-reacted DNA</td>
<td>51</td>
</tr>
<tr>
<td>4. The Absorption Spectrum of Ethidium Bromide and Ethidium Bromide-reacted DNA at Different DNA Concentrations</td>
<td>52</td>
</tr>
</tbody>
</table>
CHAPTER I

INTRODUCTION

Prior to 1965 the interactions of polycyclic aromatic hydrocarbons with DNA (Deoxyribonucleic acid) had been but moderately studied. The summarized results of these studies may be found in an excellent review by Brookes (5). It was concluded that, although a controversy existed, an apparent interaction occurred between DNA and certain aromatic hydrocarbon molecules and that the interaction was not an artifact of the reaction system.

The controversial aspects arose through in vitro studies by several investigators [Boyland and Green (4) and Giovanella, McKinney and Heidelberger (8)] and is extensively reviewed by Boyland (3) and to a lesser extent by Pullman (20). Briefly, the controversy consisted of the following: Booth and Boyland (2) found that when the aromatic hydrocarbon 3,4-benzpyrene reacted with DNA, visible spectroscopic shifts occurred and that these shifts were similar to those obtained when complexes of purines and either dibenzocarbazoles or
dibenzacridines were formed. Consistent with these data were those of Liquori et al. (17) which stated that the degree of interaction of the aromatic hydrocarbon with DNA was dependent upon the extent of denaturation of the DNA structure.

However, Heidelberger (11) and Giovanella et al. (8) demonstrated that both 3,4-benzpyrene and 1,2,5,6-dibenzanthracene were removable from DNA solutions by either centrifugation or ultrafiltration. These investigators concluded that the DNA acted to stabilize the hydrocarbon as a colloid and that no true interaction existed.

A further investigation by Boyland and Green (4) indicated that the hydrocarbons studied had a greater affinity for the polymer centrifuge tubes than for the DNA, and that when these tubes were replaced either by glass or quartz tubes the complex was stable to centrifugation.

More recently, although discrepancies still exist, there appears to be some convergence of opinion, and more unambiguous results have emerged. The findings by Ball, McCarter and Smith (1) have revealed that (1) sucrose gradient centrifugation of 3,4-benzpyrene--DNA complexes failed to separate them into component parts, (2) lower pH values enhanced the hydrocarbon binding to DNA and
the absorbance loss, upon heating, at 395.0 nm correlated well with the \( T_m \) (melting temperature) of control DNA. These data add credence to the view that a moderately strong interaction occurs. Furthermore, the existence of dichroic spectra for aromatic-hydrocarbon-reacted DNA also suggested a firm binding, possible by an intercalation mechanism such as proposed by Lerman \((15)\) for acridine dyes.

Investigations by Isenberg and Baird \((12)\) led to the conclusion that the hydrocarbons phenanthrene, pyrene, and 3,4-benzpyrene complexed with DNA but not with poly A (polyadenylate) and that the degree of complex formation varied as a function of the molecular dimensions of the hydrocarbons. In addition, spectral studies by Lesko et al. \((16)\) of the visible absorption patterns of 3,4-benzpyrene in several solvent systems added support to the intercalation model. Furthermore, these investigators, using exchange techniques, concluded that neither 3,4-benzpyrene nor 1,2-benzpyrene bind to specific and identical sites on the DNA molecule.

In vivo observations by DeMaeyer-Guigard and DeMaeyer \((6)\) that viral DNA replication was inhibited by aromatic hydrocarbons suggested a possible DNA effect similar to the action of actinomycin D.
Additional evidence by Jensen et al. (13) that 7,12-dimethyl-1,2-benzanthracene selectively damaged mammalian germinal cells which were synthesizing DNA and that the incorporation of tritiated thymidine into DNA was depressed in the presence of this hydrocarbon further suggested an effect on the DNA level. These data were extended by Loeb and Gelboin (18) with the findings that both 3,4-benzpyrene and 3-methylcholanthrene stimulated microsomal enzyme activity in rat tissue and that this stimulation was inhibited by actinomycin D. Kersten (14) demonstrated that actinomycin D interacted specifically with the guanine moiety of DNA. The specific bonding arrangement was elucidated by Hamilton (10) and was found to consist of the formation of specific hydrogen bonds between the phenoxazine ring of actinomycin D and nitrogens N1 and N3 of the guanine moiety. Further studies by Gelboin and Klein (7) indicated that treatment of mice with actinomycin D prior to the application of 7,12-dimethyl-1,2-benzanthracene significantly reduced the number of tumors induced by the hydrocarbon. These results indirectly indicated a hydrocarbon-DNA binding since actinomycin D reacts with DNA.

Finally, recent studies by Goshman and Heidelberger (9) have led to the isolation of hydrocarbon-bound DNA
from mouse skin which had been treated in vivo. These investigators studied the following hydrocarbons—9,10-dimethyl-1,2-benzanthracene, 1,2,5,6-dibenzanthracene, 1,2,3,4-dibenzanthracene, and 3-methylcholanthrene—and confirmed binding to DNA by radiochemical means. They, furthermore, concluded that the binding reaction was independent of DNA replication.

In view of the information discussed above, the following proposition was investigated: if binding reactions which occur in vitro occur in vivo, as would appear to be the case, then what would be the result of subsequent DNA replication on DNA composition?
CHAPTER BIBLIOGRAPHY


18. Loeb, L. A. and H. V. Gelboin, "Stimulation of Amino Acid Incorporation by Nuclear Ribonucleic Acid from

CHAPTER II

MATERIALS AND METHODS

Hydrocarbon Purification

The aromatic hydrocarbons, 1,2-benzanthracene, 1,2,5,6-dibenzanthracene, 3-methylcholanthrene, and 3,4-benzpyrene, were obtained from Aldrich Chemical Corporation in crystalline form. Each hydrocarbon was then dissolved in a minimal volume of anhydrous diethyl ether and refluxed under reduced pressure for two hours. The solutions were then diluted with an equal volume of diethyl ether, and 10 grams of "Norite 60" were added; reflux was then continued for an additional two hours. The "Norite 60" was removed by filtration through Whatman No. 1 filter paper, the solution concentrated to 2/3 volume by distillation and the hydrocarbons precipitated at -40°C. The precipitated product was separated by decantation and redissolved in a minimal volume of diethyl ether in a 1500 ml separatory funnel. An equal volume of distilled water was added and the mixture was shaken for thirty minutes. After phase separation, hydrocarbon crystals were present at the interface and were
removed by filtration. The hydrocarbons were dried under vacuum and stored in amber bottles until used.

Ethidium Bromide Purification

Ethidium bromide was purchased from California Biochemical Corporation and was dissolved in a 70% (v/v) methyl alcohol:HOH solution. The solution was vacuum-refluxed for thirty minutes and precipitated by rapid cooling in liquid nitrogen. The precipitate was collected by filtration and three additional recrystallizations were performed in 70, 60, 50% methyl alcohol solutions, respectively. The purity of the resulting precipitate was determined by melting-point determination and by CHN analysis.

In Vivo Hydrocarbon-DNA-Reaction System

Colloidal suspensions of the appropriate hydrocarbon were prepared as follows: 10.0 mg of the hydrocarbon were dissolved in 5.0 ml of redistilled dimethylsulfoxide (Matheson, Coleman and Bell), sterilized by passing through a 0.33 µ Millipore filter, and dispersed by rapid injection of the solution into 495.0 ml of sterile 3.0% tryptic soy (TS) broth (Difco). A sterile magnetic stir bar was introduced and the mixture stirred for ten minutes at room
temperature. The hydrocarbon suspensions were inoculated by washing and decanting 1.0 ml aliquots from stock culture slants of *Escherichia coli* B (ATCC 11303). The inoculated cultures were incubated at 37°C with slow stirring on an insulated magnetic stirrer.

**In Vivo Ethidium Bromide-DNA-Reaction System**

Purified ethidium bromide was dissolved in 3.0% tryptic soy broth to a final concentration of 20 μg/ml and the media sterilized by filtration through a 0.33 μ Millipore filter. Following filtration a sterile magnetic stir bar was added and the media innoculated and incubated as described above.

**Growth Assays**

Growth in hydrocarbon or ethidium bromide-treated and non-treated (control) systems was defined as an increase in absorbance (660.0 nm) as a function of time. In each instance, triplicate measurements on triplicate samples were performed and the mean value, with a standard deviation bar, plotted.

The assays were implemented by removing 5.0 ml of media for each measurement, harvesting the cells by centrifugation at 4°C. and triplicate equal volume washing with diethyl ether. The washed cells were then resuspended in 5.0 ml of distilled water and the absorbance at 660.0 nm recorded.
Each growth assay was treated as an independent measurement and innocula of 0.50 absorbance units used.

Preparation of DNA

The cells were harvested by centrifugation in the cold and extracted with 10.0 ml of peroxide-free diethyl ether until no absorbance could be detected at the appropriate wavelength. The cells were then washed once with 30.0 ml of 0.15 M NaCl containing 0.1 M EDTA (ethylene diamine tetraacetic acid), pH 8.0, and resuspended in 10.0 ml of NaCl-EDTA. The DNA was extracted by the method of Marmur (4) with the following modifications: (1) 1.0 ml of a 10.0 mg/ml solution of self-digested pronase (Sigma Chemical Company) was added immediately following the addition of 1.0 ml of a 30% solution of sodium lauryl sulfate, (2) the DNA was extracted with an equal volume of freshly-distilled, water-saturated phenol followed by two extractions with three volumes of diethyl ether, (3) the DNA was precipitated with 0.6 volumes of n-propyl alcohol and dissolved in either 0.02 M NaCl- 0.001 M Tris (2-amino-2-(hydroxy-methyl)-1,3-propanediol) buffer, pH 7.0 or 0.01 M NaCl- 0.0015 M trisodium citrate, pH 7.5.
Purification of Commercial DNA

Calf thymus DNA from Lot No. 58B-1420 was obtained from Sigma Chemical Company and dissolved to a final concentration of 2.0 mg/ml in 0.01 M NaCl-0.0015 M trisodium citrate. Complete dissolution usually required four days at 4°C., with gentle stirring. After dissolution, the DNA was extracted twice with an equal volume of freshly distilled, water-saturated phenol and the aqueous phase extracted three times with diethyl ether. The DNA was then precipitated with 0.6 volumes of n-propyl alcohol and the DNA dissolved in either NaCl-trisodium citrate or NaCl-Tris buffer.

In Vitro Hydrocarbon-DNA-Reaction System

Immediately prior to the start of the reaction, a one-half volume quantity of the appropriate buffer was rapidly injected with 5.0 ml of diethyl ether containing 2.0 mg of hydrocarbon. The buffer was then purged free of ether by bubbling with buffer-saturated nitrogen gas. The remaining one-half buffer volume, containing twice the required (w/v) DNA concentration, was then added rapidly and the mixture shaken at 4°C. for four days. The non-reacted colloidal hydrocarbon was removed by filtration through two layers of Whatman No. 1 filter paper.
In Vitro Ethidium Bromide-DNA-Reaction System

Purified-ethidium bromide-DNA reactions were prepared analogous to hydrocarbons-DNA reactions with the following exceptions: (1) a final concentration of 10.0 μg/ml of ethidium bromide was used and (2) the diethyl ether step was omitted since ethidium bromide is water soluble.

Measurement of Absorption Spectra

All spectra were measured on a Coleman-Hitachi 124 recording UV-Visible spectrophotometer and rechecked at random for photometric accuracy on a Cary 14 spectrophotometer.

Optically matched 1.00 cm quartz cuvettes were cleaned prior to each determination by a fifteen second immersion in boiling nitric acid followed by automatic rinsing in each of the following: (1) 500 ml glass distilled water, (2) 100 ml anhydrous methyl alcohol, and (3) 100 ml acetone. The cuvettes were then dried in a stream of nitrogen and stored in a vacuum desiccator until used.

Immediately prior to use, the spectrophotometer monochromator compartment was purged with dry nitrogen gas for thirty minutes and the band pass adjusted to 1.0 micron. The wavelength accuracy was determined by adjusting the
diffraction grating control to the 656.1 nm deuterium emission and the absorbance calibrated using a 10.0 transmittance filter.

The spectra of native DNA samples were determined at DNA concentrations of approximately 40 µg/ml in 0.02 M NaCl- Tris buffer at pH 7.0.

All hydrocarbon-DNA-reacted spectra were determined at DNA concentrations of approximately 400 and 800 µg/ml and at very low recorder input voltages (1.0 to 5.0 mv). On occasion the signal-to-noise ratios were high and the following method was necessary to retrieve the signal. Both the maximum and minimum incremental changes in absorbance for a minimum of five repetitive scans from 500.0 nm to 310.0 nm, at 2.0 nm intervals, were plotted as separate functions. The mean value for each interval was then calculated and a "best fit" polynominal was generated by a computer technique (see appendix). The resulting equation was then used to calculate the absorption spectrum.

Differential Spectra

The measurement of differential spectra consisted of placing an equally absorbing volume of control DNA in the sample beam of the spectrophotometer and recording the
spectrum. DNA concentrations of 500 µg/ml were used in each case with the exception of ethidium bromide. The large extinction coefficient of ethidium bromide allowed the use of 80 µg/ml DNA. In order to enhance photometric accuracy, the absorbance zero base was shifted to 0.4 absorbance units following initial absorbance balancing.

Melting-Temperature (Tm) Measurements

The apparatus for the measurement of DNA melting temperatures was designed, constructed, and calibrated by the author. The basic equipment consisted of jacketed optically matched quartz cuvettes, thermometer-well-heat exchanger, high-temperature circulator, thermal programmer, and a Coleman-Hitachi 124 recording spectrophotometer. When placed in the cuvette well, the thermometer-heat exchanger acted as a receiver for the measuring thermometer or the calibrating "Iron-Constantan" thermocouple. All calibrations were with respect to ice point references.

Actual Tm measurements consisted of diluting DNA samples with either NaCl-Tris or 0.01 M NaCl-0.0015 M Na3citrate buffer to a final absorbance of 0.4 units. An equal volume of buffer was placed in the reference beam of the spectrophotometer, the temperature adjusted to 25.0°C., and
The absorbance recorded. The temperature was then rapidly raised to 50.0°C. and the "Teflon" cuvette stoppers secured by twisting. Temperature increases were then either performed manually in 1.0°C. increments or linearly programmed until no further change in absorbance was observed. The data obtained from each T_m determination were then corrected for thermal expansion, converted to A_{25} (absorbance at the observed temperature relative to 25.0°C.), the minimum and maximum values found, and the T_m determined. By definition (3), the T_m value for a DNA sample corresponds to one-half the increase in total relative absorbance. Lastly, by equation number 1 of Schildkraut and Lifson (5), the guanine-cytosine content (%GC) of the DNA sample was computed. All of the above computations were performed with the computer program listed in the appendix.

\[ GC = 2.44 \left( T_m - 78.3 - 16.6 \log M \right) \]  

where M is the molar concentration of the cation.

Spectral Analysis of Native DNA (ANDS - 1)

The method used was essentially that of Hirschman and Felsenfeld (2), which was adapted for computer solution. The techniques discussed below allowed the calculation of DNA base composition as mole fraction of adenine-thymine (AT)
base pairs. Fundamentally, the determination of the mole fraction AT pairs consisted of accurately recording the absorption spectrum of a purified DNA sample as discussed on the previous page and solving the following equation:

\[ \Delta A_i = \alpha_i X + \beta_i Y + \gamma_i Z, \]

which is a linear equation in three unknowns, and

\[ \alpha_i = (1 - 2 K_i) (\epsilon_{AA_i} + \epsilon_{GG_i}), \]
\[ \beta_i = 2 [K_i (\epsilon_{AA_i} + \epsilon_{GG_i}) - \epsilon_{GG_i}], \]
\[ \gamma_i = \epsilon_{GG_i}, \]
\[ X = C (\phi^2 + \delta \phi), \]
\[ Y = C \phi, \]
\[ Z = C. \]

The value \( \Delta A_i \) is the absorbance of the DNA sample at wavelength \( i \), \( C \phi \) is the molar nucleotide concentration in moles/liter, and \( \phi \) is the mole fraction AT pairs.

The actual solution for \( C \phi \) and \( C \) required the formation of the sums

\[ \mu_1 = \sum \alpha_i \alpha_i, \mu_2 = \sum \alpha_i \beta_i, \mu_3 = \sum \alpha_i \gamma_i \]

where \( \alpha_i, \beta_i, \gamma_i \) were determined by "least squares fit" from pre-existing extinction coefficients for known DNAs. Also, six additional constants \( (S_1 \ldots S_6) \) were determined from matrix equations such as those on the following page.
where the S constants are the determinantal solutions for 
X, Y and Z. Both the \( \alpha_i, \beta_i, \gamma_i \) and the S constants 
were stored as constant data in the computer program and re-
trieved for each solution. Thus, from the stored constant 
data and the sums \( \mu_1, \mu_2 \) and \( \mu_3 \) equations 4, 5, and 6 were 
solved for molar nucleotide concentration, \( C\phi \), and mole 
fraction AT pairs.

\[
\begin{align*}
C\phi &= \mu_1 S_1 + \mu_2 S_2 + \mu_3 S_3, \quad (4) \\
C &= \mu_1 S_4 + \mu_2 S_3 + \mu_3 S_5, \quad (5) \\
\phi &= C/\phi. \quad (6)
\end{align*}
\]

Viscosity Measurements

All viscosity measurements were performed with a 
Beckman "low shear viscometer" of the Zimm and Crothers (7) 
design. With the exception of the magnetic field drive, this 
instrument is of the typical rotating cylinder viscometer 
design. Immediately prior to use, both the rotor and the 
stator were cleaned with chromic acid cleaning solution, 
carefully rinsed with glass distilled water, and kept wet
with buffer until use. Samples of DNA, 25 μg/ml in NaCl-Tris buffer, were carefully pipetted into the stator compartment until the rotor floated to a predetermined level. The complete viscometer was equilibrated to 25° C. and the magnetic drive started. In every case the rotor was allowed to make three revolutions before timing was started. At the end of five revolutions the time was recorded and the temperature checked. All measurements were performed in triplicate and the mean value determined.

The results were reported as average shear stress in the annulus in accordance with the following derivation:

Fig. 1—A generalized rotating cylinder viscometer
In order for the following relationships of the viscometer shown in Figure 2 to hold true, certain assumptions must be met:

1. The liquid must be incompressible.
2. There must exist laminar flow.
3. The streamlines of flow with respect to the horizontal plane must be perpendicular to the axis of rotation.
4. All time derivatives must be equal to zero.
5. There must be zero relative motion between the rotor and the stator.
6. Motion must occur in two dimensions only.
7. The system must be isothermal.

Given that the cylindrical equation of continuity of Bird et al. (1) is

\[ \frac{\partial \rho}{\partial t} + (\gamma / r) \left[ \partial (\rho v r \phi) / \partial r \right] + (\gamma / r) \left[ \partial (\rho v \phi) / \partial \phi \right] + \partial (\rho v z) / \partial z = 0 \]  

(7)

where

- \( r \) = relaxation time (\( \tau / G \)),
- \( \tau \) = time (seconds),
- \( r \) = radial distance measured from center,
- \( v \) = velocity component,
- \( \phi \) = angular component,
- \( p \) = density.

Then from the assumptions

\[ (\rho / r) \left( \frac{dv \phi}{d\phi} \right) = 0. \]  

(8)

Therefore, the velocity component, \( v \phi \), is a constant and the equation of motion \((1/r^2) \left( \frac{d(r s_{r \phi})}{dr} \right) = 0\)  

integrating \[ \int \left( \frac{1}{r^2} \right) \left( \frac{d(r s_{r \phi})}{dt} \right) = r^2 s = c \]  

(10)

and \( s_{r \phi} = s \) since there remains only one stress component since torque = area x radius x shear stress or, \( M = 2 \pi rh s \).  

(11)
but from equation number ten, \( c_1 = r^2s \), therefore, division by \( c_1 \) gives

\[
r^2h = M/2\pi h, \tag{12}
\]

and for any constant moment in steady state (6)

\[
M = s_b 2\pi R_b^2 h = s_c 2\pi R_c^2 h
\]

and

\[
s_b/s_c = R_c^2/R_b^2 = \epsilon^2 \tag{13}
\]

which states that the stress ratio varies inversely with the square of the radii of the rotor and the stator.

Where there exists a velocity gradient, \( \frac{dv}{dr} \), then

\[
\frac{dv}{dr} = \omega + r \frac{d\omega}{dr}. \tag{14}
\]

From equations eleven and fourteen,

\[
-r \frac{d\omega}{dr} = f(M/2\pi r^2 h) = f(s). \tag{15}
\]

Differentiation of fifteen and substitution for \( M \) gives

\[
\frac{dr}{r} = -\frac{ds}{2s} \tag{16}
\]

and

\[
\frac{d\omega}{s} = \frac{1}{2} f(s) \frac{ds}{s}. \tag{17}
\]

Integrating equation sixteen from the rotor to the stator,

\[
s = s_b, \ s = s_c \text{ and from } \omega = 0 \text{ to } \Omega (\text{angular velocity})
\]

\[
\Omega = \frac{1}{2} \int_{s_b}^{s_c} f(s) \frac{ds}{s}. \tag{18}
\]

Since no relative motion occurs between the rotor and the stator, the shear stress at the surface of the stator is zero and equation eighteen may be rearranged to give

\[
\Omega = \frac{\pi}{2} \int_{s_b}^{s_c} f(s) \frac{ds}{s} \tag{19}
\]
which is then differentiated to give

\[ f(s_b) = -2\frac{d\alpha}{d\ln s_b}, \quad (20) \]

and

1. \( f(s_b) \) is found by plotting \( \alpha \) against \( \ln s_b \), and
2. validity is established only for solutions having no yield value.

Finally, for a viscometer of narrow rotor to stator separation and from equation thirteen, the following approximation may be made:

\[ \langle s \rangle = \frac{(R_c^2 + R_b^2)/R_c^2 - R_b^2}{R_c^2 + R_b^2} \quad (21) \]

where \( \langle s \rangle \) is the mean rate of shear.

For the experiments discussed below was equal to the speed of the rotor in revolutions per second and \( R_c = 0.65 \) cm and \( R_b = 0.50 \) cm.
CHAPTER BIBLIOGRAPHY


CHAPTER III

RESULTS

The results obtained for the four separate hydrocarbons (1,2-benzanthracene, 1,2,5,6-dibenzanthracene, 3-methylcholanthrene, and 3,4-benzpyrene) and ethidium bromide under in vivo growth conditions are given in Figure 2 (See appendix II for Figs. 2, 3, 4). These data represent the results of four separate experiments in triplicate sets. Although not presented, the results of statistical analysis indicate the data to be within the tolerances of the experimental method. Also, since there exists very close agreement between the family of curves for both the dimethylsulfoxide and the dimethylsulfoxide-control, it may be concluded that dimethylsulfoxide exhibits little or no effect on Escherichia coli B growth in the concentrations used. The observed effects may, therefore, be attributed to either the hydrocarbons or the ethidium bromide.

The data obtained for DNA-hydrocarbon binding under in vitro conditions, Figure 3, is in agreement with the findings of Liquori et al. (2) for 3,4-benzpyrene. Furthermore, it
may be seen that although little binding occurs for 1,2-benzanthracene there occurs an increase for 1,2,5,6-dibenzanthracene, 3-methylcholanthrene and 3,4-benzpyrene.

Unless otherwise stated all in vitro data presented are for Escherichia coli B.

In addition to DNA binding there is an observed shift, Table I, toward longer wavelengths at the major absorption bands of the hydrocarbons when compared to spectra obtained in cyclohexane.

**TABLE I**

ULTRAVIOLET ABSORPTION PEAKS OF AROMATIC HYDROCARBONS

<table>
<thead>
<tr>
<th>Hydrocarbon:Solvent System</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-benzanthracene:</td>
<td></td>
</tr>
<tr>
<td>cyclohexane</td>
<td>342</td>
</tr>
<tr>
<td></td>
<td>358</td>
</tr>
<tr>
<td></td>
<td>388</td>
</tr>
<tr>
<td>1,2-benzanthracene:DNA</td>
<td>350</td>
</tr>
<tr>
<td></td>
<td>363</td>
</tr>
<tr>
<td></td>
<td>393</td>
</tr>
<tr>
<td>1,2,5,6-dibenzanthracene:</td>
<td></td>
</tr>
<tr>
<td>cyclohexane</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>333</td>
</tr>
<tr>
<td></td>
<td>348</td>
</tr>
<tr>
<td>1,2,5,6-dibenzanthracene:</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>331</td>
</tr>
<tr>
<td></td>
<td>340</td>
</tr>
<tr>
<td></td>
<td>360</td>
</tr>
<tr>
<td>3-methylcholanthrene:</td>
<td></td>
</tr>
<tr>
<td>cyclohexane</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>372</td>
</tr>
<tr>
<td></td>
<td>401</td>
</tr>
</tbody>
</table>
TABLE I Continued

<table>
<thead>
<tr>
<th>Hydrocarbon:Solvent System</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-methylcholanthrene:DNA</td>
<td>+ 380 412</td>
</tr>
<tr>
<td>3,4-benzpyrene:</td>
<td></td>
</tr>
<tr>
<td>cyclohexane</td>
<td>348 363 385</td>
</tr>
<tr>
<td>3,4-benzpyrene:DNA</td>
<td>355 372 395</td>
</tr>
</tbody>
</table>

+No absorption.

Also, Figure 4 indicates the existence of an isobestic point at 510 nm for ethidium bromide reacted DNA; whereas, no isobesticity was detected in hydrocarbon reacted DNAs.

The use of differential spectrophotometry (1) allows the nullification of any contribution to the spectral pattern which the solvating molecule may make. It also, under carefully controlled conditions, enhances photometric precision in addition to the gain in accuracy. When both ethidium bromide and hydrocarbon reacted-DNAs were examined differentially, with respect to DNA, Table II, it was found that a shift towards longer wavelengths still existed, but that these shifts were somewhat less than those observed from simple absorption spectra.
## TABLE II

Differential Ultraviolet Absorption Peaks for Ethidium Bromide and Aromatic Hydrocarbons

<table>
<thead>
<tr>
<th>Compound:Solvent System</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethidium bromide:NaCl-Tris</td>
<td>+</td>
</tr>
<tr>
<td>Ethidium bromide:DNA</td>
<td>+</td>
</tr>
<tr>
<td>1,2-benzanthracene:cyclohexane</td>
<td>342</td>
</tr>
<tr>
<td>1,2-benzanthracene:DNA</td>
<td>345</td>
</tr>
<tr>
<td>1,2,5,6-dibenzanthracene:DNA</td>
<td>320</td>
</tr>
<tr>
<td>1,2,5,6-dibenzanthracene:DNA</td>
<td>326</td>
</tr>
<tr>
<td>3-methylcholanthrene:cyclohexane</td>
<td>+</td>
</tr>
<tr>
<td>3-methylcholanthrene:DNA</td>
<td>+</td>
</tr>
<tr>
<td>3,4-benzpyrene:cyclohexane</td>
<td>348</td>
</tr>
<tr>
<td>3,4-benzpyrene:DNA</td>
<td>354</td>
</tr>
</tbody>
</table>

*+No absorption.*

Both simple absorption spectra and differential spectra were obtained for DNAs extracted from cells grown in the presence of both ethidium bromide and polycyclic hydrocarbons. While DNA preparations from ethidium bromide-grown cells
showed slight amounts of bound dye, it was impossible to obtain bound DNA from hydrocarbon-grown cells.

The results of in vitro thermal denaturation, $T_m$, and rate of shear, $\langle s \rangle$, studies are summarized in Table III and indicate that increases in both $T_m$ and $\langle s \rangle$ occur following binding. These increases are the greatest for ethidium bromide and the least for 1,2-benzanthracene.

**TABLE III**

$T_m$ and $\langle s \rangle$ VALUES FOR IN VITRO AROMATIC HYDROCARBON AND ETHIDIUM BROMIDE REACTED DNAs IN 0.02 M NaCl-Tris

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of Observations</th>
<th>$T_m$ (°C.)</th>
<th>$\langle s \rangle$ dynes/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>75.0.3</td>
<td>382</td>
</tr>
<tr>
<td>Ethidium Bromide</td>
<td>7</td>
<td>87</td>
<td>597</td>
</tr>
<tr>
<td>1,2-benzanthracene</td>
<td>5</td>
<td>76</td>
<td>396</td>
</tr>
<tr>
<td>1,2,5,6-dibenzanthracene</td>
<td>7</td>
<td>77</td>
<td>411</td>
</tr>
<tr>
<td>3-methylcholanthrene</td>
<td>5</td>
<td>77.9</td>
<td>425</td>
</tr>
<tr>
<td>3,4-benzpyrene</td>
<td>9</td>
<td>79</td>
<td>440</td>
</tr>
</tbody>
</table>
The analysis, however, of in vivo prepared DNAs for $\%GC$ by $T_m$ measurements, mole fraction AT pairs by ANDS - 1, and viscosity indicate the following: (1) a decrease in the $\%GC$ concentration, (2) an increase in the mole fraction AT pairs, and (3) a decrease in the $\langle\phi\rangle$. These observations are summarized in Table IV.

**TABLE IV**

**THE IN VIVO EFFECTS OF AROMATIC HYDROCARBONS AND ETHIDIUM BROMIDE ON THE CHARACTERISTICS OF ESCHERICHIA COLI DNA**

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of observations</th>
<th>$%GC$</th>
<th>Mole Fraction AT Pairs</th>
<th>$\langle\phi\rangle$ dynes/cm$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>48</td>
<td>51</td>
<td>390</td>
</tr>
<tr>
<td>Ethidium bromide</td>
<td>8</td>
<td>41</td>
<td>59</td>
<td>253.5</td>
</tr>
<tr>
<td>1,2-benzanthracene</td>
<td>8</td>
<td>48</td>
<td>52</td>
<td>387</td>
</tr>
<tr>
<td>1,2,5,6-di-benzanthracene</td>
<td>6</td>
<td>47.5</td>
<td>53</td>
<td>382</td>
</tr>
<tr>
<td>3-methylcholanthrene</td>
<td>5</td>
<td>46.8</td>
<td>55</td>
<td>384</td>
</tr>
<tr>
<td>3,4-benzpyrene</td>
<td>13</td>
<td>45.6</td>
<td>56</td>
<td>271</td>
</tr>
</tbody>
</table>
CHAPTER BIBLIOGRAPHY


CHAPTER IV

DISCUSSION

The first suggestion for an interaction between polycyclic aromatic hydrocarbons and DNA was made in 1953 by Booth and Boyland (1). In a later study Boyland and Green (2) proposed an intercalation mechanism similar to that proposed by Lerman (11). There remained, however, a controversy between Giovanella et al. (5) and Heidelberger (8) with respect to the methods used and the interpretation of the results.

In an attempt to resolve the difficulties, Boyland and Green (2) and Nagata et al. (14) performed additional studies using different techniques and appeared to establish the binding effect. Additional evidence was supplied later by Isenberg and Baird (10) and by Lesko and his co-workers (12).

The data presented in Figure 3 of this paper further support the interaction proposition. It would also seem possible to rate the degree of interaction as follows: 1,2-benzanthracene < 3-methylcholanthrene < 1,2,5,6-dibenzanthracene < 3,4-benzpyrene.
In view of the spectral shifts towards longer wavelengths (Tables I and II), the possibility of a "charge transfer" type mechanism, as developed by Pullman and Pullman (15) and Hoffman and Ladik (9), should be considered. It is noted, however, that spectral shifts alone do not constitute proof for a "charge transfer" mechanism but merely suggest it as a possibility.

The observations in Figure 4, that ethidium bromide-reacted-DNA contains an isobestic point, confirm the findings of Waring (16). The existence of an isobestic point suggests that the observed spectra are a result of two forms of ethidium bromide, i.e., a free and a bound form. The lack of isobesticity for hydrocarbon-reacted DNAs implies that either the spectral contribution is due to a bound form or that any free hydrocarbon lies below the limit of instrumental capability. In view of the extremely low aqueous solubilities of the hydrocarbons studied, the latter may well be the case. Nevertheless, it would appear that there exists an interaction between polycyclic aromatic hydrocarbons and DNA and that this interaction is amenable to spectrophotometric investigation.

Viscosity determinations also present data confirming hydrocarbon binding to DNA. The results summarized in
Table III indicate an increase in the mean rate of shear, \langle s \rangle, for both hydrocarbon and ethidium bromide reacted DNAs. These data would indicate that following binding there is an overall increase in the rigidity of the DNA molecule which would therefore indirectly lend support to the intercalation concept (11).

Obviously, information obtained \textit{in vitro} from isolated systems cannot necessarily be extrapolated to \textit{in vivo} systems without extensive investigation. Using isotopic methods, Brookes and Lawley (3) and, more recently, Goshman and Heidelberger (6) have been able to isolate hydrocarbon-labeled DNA from the skin of higher animals.

It was first suspected that if certain hydrocarbons would react \textit{in vivo} with DNA then perhaps one of the manifestations of the reaction would be an alteration in growth kinetics. The data presented in Figure 2 indicate that some effect is observed but that the magnitude of the effect is small. That this statement is valid is especially relevant when the data are compared to that of ethidium bromide. It would appear that ethidium bromide has an immediate suppressive effect on the growth of \textit{Escherichia coli} B and that this effect is of several orders of magnitude greater than that of hydrocarbons.
The most interesting results obtained in this study are those summarized in Table IV. These data indicate that the DNA isolated from cells grown in the presence of certain polycyclic hydrocarbons is altered with respect to its nucleotide composition. This alteration is reflected in the decrease in the $GC$ value and a concomitant increase in the mole fraction $AT$ pairs. At this point it is important to note that there exists an exact correlation between the extent of hydrocarbon bound in vitro and the decrease in $GC$ value in vivo. That is, the magnitude of the $GC$ decrease varied directly with the hydrocarbon present in the growth medium in the order $1,2$-benzanthracene $< 3$-methylcholanthrene $< 1,2,5,6$-dibenzanthracene $< 3,4$-benzpyrene. As would be expected, the increase in mole fraction $AT$ pairs was consistent with the same series.

Although somewhat disappointing, the mean rate of shear data for in vivo systems reflect differences when compared to control values. The most important results are, however, that an increase in $\langle e \rangle$ exists for in vitro systems and a decrease exists for in vivo systems. As stated above, there would appear to be a loss in molecular rigidity, and this loss is greater than control values for in vivo systems.
The data obtained for \textit{in vivo} ethidium bromide reactions agree qualitatively with the effects of hydrocarbons on DNA. Quantitatively, however, the difference is one of magnitude.

In view of the information discussed above, it would appear that a discrete relationship exists between certain polycyclic aromatic hydrocarbons, ethidium bromide, and DNA. Under \textit{in vivo} conditions these compounds are able to influence the replication of the DNA. Furthermore, these influences are permuted towards increases in AT pair concentrations. Since neither hydrocarbon nor ethidium bromide-bound DNA was isolated from \textit{in vivo} systems to any appreciable extent, it is suggested that perhaps weak binding forces were destroyed during extraction and the DNA-bound compounds removed. It is also possible that, since protein binding is known to occur (13) and to be quite extensive, dilution of the hydrocarbon by protein binding occurred as a function of incubation time.

Since there occurs an increase in AT pair concentration, it is suggested that perhaps GC pairs are attacked preferentially by the hydrocarbons or at least are more subject to their reactivity. This view is reinforced by the findings of Gelboin and Klein (4) that tumor induction is substantially inhibited in mice by pre-treatment with actinomycin D, which
has been shown to react with guanine residues in DNA (7). Regardless of the binding site, there remains the possibility that during replication of hydrocarbon-bound DNA, the template induces a replication or copy error by presenting a topographical configuration which more closely resembles an AT pair than a GC pair and an error is copied in.

Whatever the mechanism involved may be, there appears to be a definite DNA alteration, and this alteration results in a decrease in GC pairs and an increase in AT pairs.


APPENDIX I

The below-listed programs contain the necessary steps for (1) computation of the necessary generator equations for spectral curve fitting, (2) calculation of $T_m$ and %GC values and (3) computing the mole fraction AT base pairs for any given DNA molecule.

All of these listed programs were designed to be used on IBM (International Business Machines) 1620, 20k computer, equipped with either AFIT or PDQ "FORTRAN" with format language.
C POLYNOMIAL CURVE FIT FOR UP TO 5TH ORDER.  

C SS3 -- ON TO PRINT BACK SOLUTIONS AFTER EACH FIT.  
DIMENSION X(250), Y(250), A(7,7), SOX(15), SOY(7)  

1 READ 10, M, TOL, LAST  
10 FORMAT (13,610.,12)  
DO 40 I=1,M  
READ 11, X(I), Y(I)  
11 FORMAT (2E10.4)  
40 CONTINUE  
70 SUX(1)=0.  
SUX(2)=0.  
SUX(3)=0.  
SUY(1)=0.  
SUY(2)=0.  
DO 90 I=1,M  
SUX(2)=SUX(2)+X(I)  
SUX(3)=SUX(3)+X(I)*X(I)  
SUY(1)=SUY(1)+Y(I)  
SUY(2)=SUY(2)+Y(I)*Y(I)  
90 CONTINUE  
100 A(I,J)=SUX(IK)  
101 A(I,KK)=SUY(I)  
DO 140 I=1,L  
A(KK,J)=0.  
110 A(KK,J)=0.  
C=1./A(1,1)  
DO 120 J=2,KK  
120 A(J,J)=A(J,J)-A(1,J)*A(1,1)*C  
DO 140 J=KK+1,KK  
140 A(J,J)=A(J,J+1,J)  
S1=0.  
DO 160 J=1,L  
S1=A(1,KK)  
150 S1=S1+X(J+1,KK)*Y(J)  
160 S2=S2+(S1-Y(J))*S1-Y(J))  
S=S2/L  
165 CONTROL 971  
PRINT 204  
PRINT 205, MORD, TTL, S2,  
PRINT 206  
DO 164 I=1,L  
164 PRINT 205, J, A(I,KK)  
IF (SENSE = SWITCH 2) 167, 168  
168 IF (SENSE-LAST) 186, 167  
166 IF (S2-TOL) 167, 167, 171  
167 PRINT T 208  
171 CONTINUE  
}
S1 = A(1, KK)

DO 165 J = 1, M

168 S1 = S1 + A(J + 1, KK) * X(I) ** J

S3 = V(I) - S1

PRINT 209, X(I), Y(I), S1, S3

IF(INDRO.LT.LAST) 170, 1, 171

170 IF(S2 - TOL) 1, 1, 171

171 INDRO = INDRO + 1

J = 2 * INDRO

SU = X(I) = 0

SU = X(J + 1) = 0

SU = Y(INDRO + 1) = 0

DO 172 I = 1, I

SU = X(I) = SU = X(I) + X(I) ** (J - 1)

SU = X(J + 1) = SU = X(J + 1) + X(I) ** J

SU = Y(INDRO + 1) = SU = Y(INDRO + 1) + Y(I) * X(I) ** KRO

GO TO 91

204 FORMAT (/ 68, X, 10X, 3HTOL, 13X, 3HDEV, 4X, 3H0BS /)

205 FORMAT (13, 2E14.8, 14)

206 FORMAT (/ 8H COEFF. /)

208 FORMAT (/ 68, 14X, 17X, 14X, 17X, 24HDC, 15X, 4H0BS /)

209 FORMAT (E14.8, 4X, E14.8, 4X, E14.8, 4X, E14.8)

END
25 1.0000  
26 1.0003  
27 1.0006  
28 1.0008  
29 1.0011  
30 1.0014  
31 1.0017  
32 1.0020  
33 1.0024  
34 1.0027  
35 1.0030  
36 1.0034  
37 1.0037  
38 1.0041  
39 1.0045  
40 1.0049  
41 1.0053  
42 1.0057  
43 1.0061  
44 1.0065  
45 1.0069  
46 1.0073  
47 1.0078  
48 1.0082  
49 1.0087  
50 1.0091  
51 1.0095  
52 1.0100  
53 1.0105  
54 1.0110  
55 1.0115  
56 1.0120  
57 1.0125  
58 1.0130  
59 1.0135  
60 1.0141  
61 1.0147  
62 1.0152  
63 1.0157  
64 1.0162  
65 1.0168  
66 1.0174  
67 1.0180  
68 1.0186  
69 1.0191  
70 1.0197  
71 1.0203  
72 1.0209  
73 1.0215  
74 1.0221  
75 1.0228  
76 1.0234  
77 1.0240  
78 1.0246  
79 1.0253  
80 1.0259  
81 1.0266  
82 1.0273
<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>83</td>
<td>1.0280</td>
</tr>
<tr>
<td>84</td>
<td>1.0287</td>
</tr>
<tr>
<td>85</td>
<td>1.0293</td>
</tr>
<tr>
<td>86</td>
<td>1.0300</td>
</tr>
<tr>
<td>87</td>
<td>1.0308</td>
</tr>
<tr>
<td>88</td>
<td>1.0314</td>
</tr>
<tr>
<td>89</td>
<td>1.0321</td>
</tr>
<tr>
<td>90</td>
<td>1.0329</td>
</tr>
<tr>
<td>91</td>
<td>1.0336</td>
</tr>
<tr>
<td>92</td>
<td>1.0343</td>
</tr>
<tr>
<td>93</td>
<td>1.0351</td>
</tr>
<tr>
<td>94</td>
<td>1.0358</td>
</tr>
<tr>
<td>95</td>
<td>1.0365</td>
</tr>
<tr>
<td>96</td>
<td>1.0373</td>
</tr>
<tr>
<td>97</td>
<td>1.0380</td>
</tr>
<tr>
<td>98</td>
<td>1.0388</td>
</tr>
<tr>
<td>99</td>
<td>1.0396</td>
</tr>
<tr>
<td>100</td>
<td>1.0404</td>
</tr>
<tr>
<td>101</td>
<td>1.0411</td>
</tr>
<tr>
<td>102</td>
<td>1.0419</td>
</tr>
<tr>
<td>103</td>
<td>1.0426</td>
</tr>
<tr>
<td>104</td>
<td>1.0433</td>
</tr>
<tr>
<td>105</td>
<td>1.0441</td>
</tr>
</tbody>
</table>

99999 99999
DIMENSION T(82), VT(82), A(81), AP(81), ATA25(81), XT(81)
READ, XX

C XX - VARIABLE 1 IN GC FORMULA
DO 100 I=1, R2
   T(I) = 0
100 VT(I) = 0
   DO 1 1=1, R2
      READ, T(I), VT(I)
      IF(T(I) .EQ. 99999.)1, 200, 1
C•  i•
   200 1=0
      DO 1000 L=1, 81
         XT(L) = 0
         A(L) = 0
         AP(L) = 0
1000 ATA25(L) = 0
   2 I=I+1
      READ, T1, A1
      IF(T1 .NE. 99999.)6, 5, 6
9 CONTINUE
STOP
5 A(I) = A1
   XT(I) = T1
   AP(I) = A(I) * VT(1)
   ATA25(I) = AP(I) / A(1)
   GO TO 2
6 I=I-1
   IF(SENSE SWITCH 1)500, 501
C SSI - ON READ IN MIN READ IN MAX
C OFF READ IN INTERVAL CHANGE AND STARTING POINT
500 READ, TX1, X', TX2, X MAX
   DO 502 L=1, 81
      IF(TX1-T(L))502, 503, 502
502 CONTINUE
STOP
503 X'=X'-X1*VT(L)/AP(1)
   DO 504 L=1, 81
      IF(TX2-T(L))504, 505, 504
504 CONTINUE
STOP
505 X MAX = X'-X1*VT(L)/AP(1)
   GO TO 510
501 READ, ZXY, 199
C ZXY - INTERVAL CHANGE
C 199 - INTERVAL T1 START COMPARISON AT - 1.
   DO 7 L=108, 1
      DIFF = ABS(ATA25(L)-ATA25(L-1))
      IF(DIFF .GT. ZXY)17, 8, 9
7 CONTINUE
STOP
8 X+1 = ATA25(L-1)
   DO 9 JJ=1, 1
      J=1-JJ+1
      DIFF = ABS(ATA25(J)-ATA25(J-1))
      IF(DIFF .GT. ZXY)18, 9, 10
9 CONTINUE
STOP
10 X=AX=ATA25(I)
510 XM10=(XM:AX-XKI)/2+XKI
DO 201 LI=1,10
 IF(XM10=ATA25(LI))203,202,201
201 CONTINUE
 STOP
202 TEMP=XT(LI)
 GO TO 205
203 VX=ATA25(LI)-ATA25(LI-1)
 TEMP=XT(LI-1)+(X:ID-ATA25(LI-1))/VX*(XT(LI)-XT(LI-1))
205 PER=(AP(I)-AP(1))/AP(I)*100.
 GC=(TEMP-XX)*2.4
 PRINT20
20 FORMAT(10X,4HTEP,15X,1HEX,15X,2HA9,10X,7HAPTA25/)
 DO 21 I=1,1
21 PRINT22,XT(I),5(I),AP(I),ATA25(I)
22 FORMAT(F14.0,F16.4,F17.4,F16.4)
 PRINT 25,IX=1,IX=AX
25 FORMAT(15X,3HEX,15X,7HEXP,15X,3HEX,7F4.4)/
 PRINT23
23 FORMAT(15X,8HTEMP,15X,7HEXP,15X,8HTEMP,15X,7HEXP)/
 PRINT24,X=1,TEMP
24 FORMAT(15X,7HEPAT/A25,7F4.4/5X,4HTEMP,7F2.4)
 PRINT240,PER,GC
240 FORMAT(15X,2HGC,F4.2/5X,6HGC=F10.4)
 CGT=CGT+971.1
 GO TO 200
 END
ANALYSIS OF NATIVE DNA SPECTRA

DIMENSION S(5), A(12), B(12), G(12), X(12)

DO 2 I=1,12
READ 1, A(I), B(I), G(I)
FORMAT (3F10.0)
DO 6 I=1,5
READ 7, S(I)
S(I) = S(I) * 1.E-07
FORMAT (F7.4)
DO 3 I=1,12
READ 4, X(I)
FORMAT (F10.3)
X1 = 0
X2 = 0
X3 = 0
DO 5 I=1,12
X1 = X1 + A(I) * X(I)
X2 = X2 + B(I) * X(I)
X3 = X3 + G(I) * X(I)
C0 = X1 * S(1) + X2 * S(2) + X3 * S(3)
C = X1 * S(4) + X2 * S(3) + X3 * S(5)
O = C0 / C
C0TRIAL 071
DO 20 I=1,12
PRINT 21, X(I)
FORMAT (25X, F10.4)
PRINT 21, Xi:1, Xi:2, Xi:3, Cu, C0
8 FORMAT (5X, A1, =, E10.2, 5X, A1, =, F10.2, 5X, A1, =, E10.2, 5X, A1, =, E10.2, 5X, A1, =, E10.2)
Cu = 10
END
APPENDIX II
Fig. 2—The effect on the growth of *Escherichia coli* B of aromatic hydrocarbons; 1,2-benzanthracene, 1,2,5,6-dibenzanthracene, 3-methylcholanthrene and 3,4-benzpyrene (III) and ethidium bromide.
Fig. 3--The absorption spectra of in vitro polycyclic hydrocarbon reacted DNA, 1,2-benzanthracene (--), 1,2,5,6-dibenzanthracene (///), 3-methylcholanthrene (...) and 3,4-benzpyrene (—).
Fig. 4—The absorption spectrum of ethidium bromide and ethidium bromide reacted DNA at different DNA concentrations.
BIBLIOGRAPHY

Books


Articles


Brookes, Peter, "Quantitative Aspects of the Reaction of Some Carcinogens with Nucleic Acids and the Possible


