WHITE, Robert August, 1939-
PHOTO-REDOX REACTIONS OF CHLOROPHYLL AND
CHLOROPHYLL ANALOGS IN THE PRESENCE OF
QUINONES AND HYDROQUINONES.

The University of Arizona, Ph.D., 1971
Chemistry, biological

University Microfilms, A XEROX Company, Ann Arbor, Michigan

THIS DISSERTATION HAS BEEN MICROFILMED EXACTLY AS RECEIVED
PHOTO-REDOX REACTIONS OF CHLOROPHYLL AND CHLOROPHYLL ANALOGS IN THE PRESENCE OF QUINONES AND HYDROQUINONES

by

Robert August White

A Dissertation Submitted to the Faculty of the
DEPARTMENT OF CHEMISTRY
In Partial Fulfillment of the Requirements
For the Degree of
DOCTOR OF PHILOSOPHY
In the Graduate College
THE UNIVERSITY OF ARIZONA

1971
I hereby recommend that this dissertation prepared under my direction by Robert August White entitled Photo-Redox Reactions of Chlorophyll and Chlorophyll Analogs in the Presence of Quinones and Hydroquinones be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy.

Dissertation Director: Gordon Tollis
Date: 11/18/70

After inspection of the final copy of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:

E. K. Vennesland
Michael E. W. O. Morris
Richard S.nan
L.S. Fosbe

This approval and acceptance is contingent on the candidate's adequate performance and defense of this dissertation at the final oral examination. The inclusion of this sheet bound into the library copy of the dissertation is evidence of satisfactory performance at the final examination.
STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the library.

Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Robert A. White
ACKNOWLEDGMENTS

I wish to express my sincere gratitude to Dr. Gordon Tollin for his suggestions, encouragement, support and constructive criticisms which made this work possible. I would also like to thank Dr. Albert Frenkel of the Botany Department of the University of Minnesota for his patience and encouragement.

This work was supported in part by the U.S. Atomic Energy Commission, Contract No. AT(ll-1)908, and by the U.S. Air Force Cambridge Research Laboratories, Contract No. 19(628)4376.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF ILLUSTRATIONS</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xxiii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>xxv</td>
</tr>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Structures and Properties of Chlorophyll and Chlorophyll Analogs</td>
<td>2</td>
</tr>
<tr>
<td>Role of Chlorophyll in Green Plant Photosynthesis</td>
<td>23</td>
</tr>
<tr>
<td>Electronic Excited States of Chlorophyll</td>
<td>25</td>
</tr>
<tr>
<td>The Photo-oxidation and Photo-reduction of Chlorophyll as a Model of Photosynthetic Activity</td>
<td>29</td>
</tr>
<tr>
<td>Chlorophyll Photo-reduction</td>
<td>29</td>
</tr>
<tr>
<td>Chlorophyll Photo-oxidation</td>
<td>31</td>
</tr>
<tr>
<td>2. CHEMICAL PREPARATIONS AND INSTRUMENTATION</td>
<td>35</td>
</tr>
<tr>
<td>Chemical Preparations</td>
<td>35</td>
</tr>
<tr>
<td>Extraction of Chlorophyll a</td>
<td>35</td>
</tr>
<tr>
<td>Metal Chelates of Pheophytin, Hematoporphyrin and Phthalocyanine</td>
<td>36</td>
</tr>
<tr>
<td>Extraction of Bacteriochlorophyll</td>
<td>37</td>
</tr>
<tr>
<td>Reagents</td>
<td>37</td>
</tr>
<tr>
<td>Degassing Techniques</td>
<td>39</td>
</tr>
<tr>
<td>Instrumentation</td>
<td>41</td>
</tr>
<tr>
<td>Optical Spectroscopy during Steady Illumination</td>
<td>41</td>
</tr>
<tr>
<td>ESR Spectroscopy during Steady Illumination</td>
<td>45</td>
</tr>
<tr>
<td>Flash Spectroscopy</td>
<td>46</td>
</tr>
<tr>
<td>3. STEADY ILLUMINATION EXPERIMENTS</td>
<td>51</td>
</tr>
<tr>
<td>Experiments in Non-Viscous Solvents</td>
<td>51</td>
</tr>
<tr>
<td>Experiments in Viscous Solvents</td>
<td>68</td>
</tr>
<tr>
<td>Spectral Changes</td>
<td>69</td>
</tr>
<tr>
<td>iv</td>
<td></td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS—Continued

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinetic Comparisons and ESR Signals</td>
<td>75</td>
</tr>
<tr>
<td>Illumination Intensity</td>
<td>93</td>
</tr>
<tr>
<td>pH of Solvent</td>
<td>96</td>
</tr>
<tr>
<td>Temperature Dependence</td>
<td>103</td>
</tr>
<tr>
<td>Quenching Experiments</td>
<td>103</td>
</tr>
<tr>
<td>Effect of Porphyrin and Quinone Species on Photoreactions</td>
<td>130</td>
</tr>
<tr>
<td>4. SUMMARY OF MAJOR CONCLUSIONS FROM STEADY-STATE EXPERIMENTS</td>
<td>156</td>
</tr>
<tr>
<td>5. FLASH PHOTOLYSIS EXPERIMENTS</td>
<td>159</td>
</tr>
<tr>
<td>Flash Experiments in the Presence of Quinones</td>
<td>188</td>
</tr>
<tr>
<td>6. DISCUSSION</td>
<td>215</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>232</td>
</tr>
</tbody>
</table>
# LIST OF ILLUSTRATIONS

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Chlorophylls a and b</td>
<td>3</td>
</tr>
<tr>
<td>2.</td>
<td>Visible spectrum of chlorophyll a in diethyl ether</td>
<td>4</td>
</tr>
<tr>
<td>3.</td>
<td>Visible spectrum of chlorophyll a in CBE (cyclohexanol-tert-butanol-ethanol 675:200:125)</td>
<td>5</td>
</tr>
<tr>
<td>4.</td>
<td>Visible spectrum of chlorophyll b in diethyl ether</td>
<td>6</td>
</tr>
<tr>
<td>5.</td>
<td>Chlorophyll c</td>
<td>8</td>
</tr>
<tr>
<td>6.</td>
<td>Bacteriochlorophyll</td>
<td>10</td>
</tr>
<tr>
<td>7.</td>
<td>Visible spectrum of bacteriochlorophyll in diethyl ether</td>
<td>11</td>
</tr>
<tr>
<td>8.</td>
<td>Visible spectrum of bacteriochlorophyll in CBE</td>
<td>12</td>
</tr>
<tr>
<td>9.</td>
<td>Chlorobium chlorophyll</td>
<td>13</td>
</tr>
<tr>
<td>10.</td>
<td>Protoporphyrin IX</td>
<td>14</td>
</tr>
<tr>
<td>11.</td>
<td>Hematoporphyrin IX</td>
<td>15</td>
</tr>
<tr>
<td>12.</td>
<td>Visible spectrum of hematoporphyrin IX in pyridine-water (55:5)</td>
<td>16</td>
</tr>
<tr>
<td>13.</td>
<td>Visible spectrum of zinc-hematoporphyrin in CBE</td>
<td>17</td>
</tr>
<tr>
<td>14.</td>
<td>Zinc-phthalocyanine</td>
<td>18</td>
</tr>
<tr>
<td>15.</td>
<td>Visible spectrum of zinc-phthalocyanine in diethyl ether</td>
<td>19</td>
</tr>
<tr>
<td>16.</td>
<td>Visible spectrum of pheophytin a in CBE</td>
<td>20</td>
</tr>
<tr>
<td>17.</td>
<td>Visible spectrum of zinc-pheophytin a in CBE</td>
<td>21</td>
</tr>
<tr>
<td>Figure</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>18. Visible spectrum of cadmium-pheophytin a and b (in equilibrium with free pheophytin) in CBE</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>19. Cryostat and sample cuvette for measuring optical density</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>20. Schematic of apparatus for steady illumination of sample during optical density measurements</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>21. ESR tube for degassed samples</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>22. Schematic of flash apparatus</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>23. Cary cell and stopcock assembly for flash photolysis experiments</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>24. Five-line ESR signal of benzosemiquinone anion. Chlorophyll a ($4 \times 10^{-5}$M); benzoquinone ($1 \times 10^{-3}$M) in ethanol. Illuminated with red light at room temperature.</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>25. ESR decay curve from CAT memory and second order plot. Chlorophyll a ($4 \times 10^{-5}$M); benzoquinone ($1 \times 10^{-3}$M) in ethanol. Illuminated with red light at room temperature.</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>26. Second order plot of light-off decay for ESR signal of semiquinone anion. Chlorophyll a ($4 \times 10^{-5}$M); benzoquinone ($1 \times 10^{-3}$M) in ethanol. Illuminated with red light at -70°C to -80°C.</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>27. Six-line ESR signal of the protopated benzosemi-quinone. Chlorophyll a ($4 \times 10^{-5}$M); benzoquinone ($1 \times 10^{-3}$M) in ethanol to which 0.2 ml/10 ml glacial acetic acid was added. Illuminated with red light at room temperature.</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>28. Difference spectrum obtained on illumination of the system; chlorophyll a ($1.5 \times 10^{-5}$M); hydroquinone ($4 \times 10^{-2}$M) in pyridine-water (55:5) at -30°C to -35°C.</td>
<td>59</td>
<td></td>
</tr>
</tbody>
</table>
## LIST OF ILLUSTRATIONS--Continued

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.</td>
<td>Difference spectrum obtained on illumination of the system: chlorophyll a ((1.5 \times 10^{-5} M)); hydroquinone ((4 \times 10^{-2} M)) in ethanol at (-55^\circ) to (-60^\circ)C.</td>
<td>60</td>
</tr>
<tr>
<td>30.</td>
<td>Difference spectrum obtained on illumination of the system: chlorophyll a ((1.5 \times 10^{-5} M)); benzoquinone ((1.0 \times 10^{-2} M)) in pyridine-water (55:1) at (-29^\circ) to (-36^\circ)C.</td>
<td>61</td>
</tr>
<tr>
<td>31.</td>
<td>Second order plot of light-off decay of 430 nm band of the benzosemiquinone anion. Chlorophyll a ((1.5 \times 10^{-5} M)); hydroquinone ((4 \times 10^{-2} M)) in pyridine-water (55:1) at (-30^\circ) to (-35^\circ)C.</td>
<td>63</td>
</tr>
<tr>
<td>32.</td>
<td>Difference spectrum obtained on illumination of the system: chlorophyll a ((1.8 \times 10^{-5} M)); benzoquinone ((1.0 \times 10^{-2} M)) in ethanol at (-70^\circ) to (-90^\circ)C.</td>
<td>65</td>
</tr>
<tr>
<td>33.</td>
<td>First order plot of light-off decay of bleaching at 430 nm. Chlorophyll a ((1.8 \times 10^{-2} M)); benzoquinone ((1.0 \times 10^{-2} M)) in ethanol at about (-100^\circ)C.</td>
<td>67</td>
</tr>
<tr>
<td>34.</td>
<td>Difference spectrum obtained on illumination of the system: chlorophyll a ((2 \times 10^{-5} M)); benzoquinone ((1.0 \times 10^{-2} M)) in CBE at rm. temp.</td>
<td>71</td>
</tr>
<tr>
<td>35.</td>
<td>Difference spectrum obtained on illumination of the system: chlorophyll a ((2.0 \times 10^{-5} M)); 2,5-dichloro-p-benzoquinone ((1.1 \times 10^{-3} M)) in CBE ((N_2\ \text{purged})) at (-54^\circ) to (-63^\circ)C.</td>
<td>72</td>
</tr>
<tr>
<td>36.</td>
<td>Difference spectrum obtained on illumination of the system: chlorophyll a ((2.2 \times 10^{-5} M)); methyl-p-benzoquinone ((1.0 \times 10^{-2} M)) in CBE ((N_2\ \text{purged})), at (-40^\circ) to (-45^\circ)C.</td>
<td>73</td>
</tr>
<tr>
<td>37.</td>
<td>Difference spectrum obtained on illumination of the system: chlorophyll a ((2.1 \times 10^{-5} M)); 2,3,5-trimethyl-p-benzoquinone ((1.1 \times 10^{-2} M)) in CBE ((N_2\ \text{purged})) at (-45^\circ) to (-50^\circ)C.</td>
<td>74</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>38</td>
<td>First order plot of light-off decay for bleaching at 630 nm and ESR signal. System: chlorophyll a (2 x 10^{-5}M); 2,5-dichloro-p-benzoquinone (1.1 x 10^{-3}M) in CBE (refluxed) at rm. temp.</td>
<td>80</td>
</tr>
<tr>
<td>39</td>
<td>First order plot of light-off decay for bleaching at 670 nm and ESR signal. System: chlorophyll a (2 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (refluxed) at rm. temp.</td>
<td>81</td>
</tr>
<tr>
<td>40</td>
<td>First order plots of light-off decay for bleaching at 430 nm, rise at 460 nm and ESR signal. System: chlorophyll a (2 x 10^{-5}M); methyl-p-benzoquinone (0.9 x 10^{-2}M) in CBE (refluxed) at rm. temp.</td>
<td>82</td>
</tr>
<tr>
<td>41</td>
<td>Plot showing multiphasic character of light-on rise curve. Rise velocity (Δabsorbance/sec) versus concentration (Δabsorbance) for bleaching at 430 nm. System: chlorophyll a (2 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (refluxed), at rm. temp.</td>
<td>84</td>
</tr>
<tr>
<td>42</td>
<td>Optical light-on and light-off curves for the system: chlorophyll a (1.1 x 10^{-5}M); p-benzoquinone (2.1 x 10^{-4}M) in CBE (refluxed) at rm. temp.</td>
<td>85</td>
</tr>
<tr>
<td>43</td>
<td>Optical light-on curves for the system: chlorophyll a (1.9 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (refluxed), at rm. temp.</td>
<td>86</td>
</tr>
<tr>
<td>44</td>
<td>ESR signals obtained for steady illumination of chlorophyll-quinone systems.</td>
<td>88</td>
</tr>
<tr>
<td>45</td>
<td>ESR and optical light-on and light-off curves. System: chlorophyll a (1.9 x 10^{-5}M); 2,5-dichloro-p-benzoquinone (1.1 x 10^{-3}M) in CBE (refluxed), at rm. temp.</td>
<td>89</td>
</tr>
<tr>
<td>46</td>
<td>ESR and optical light-on and light-off curves. System: chlorophyll a (1.9 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (refluxed), at rm. temp.</td>
<td>90</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>47.</td>
<td>ESR and optical light-on and light-off curves. System: chlorophyll a (1.9 x 10^{-5}M); methyl-p-benzoquinone (0.9 x 10^{-2}M) in CBE (refluxed), at rm. temp.</td>
<td>91.</td>
</tr>
<tr>
<td>48.</td>
<td>ESR and optical light-on and light-off curves. System: chlorophyll a (1.9 x 10^{-5}M); 2,5-dimethyl-p-benzoquinone (0.6 x 10^{-2}M) in CBE (refluxed), at rm. temp.</td>
<td>92.</td>
</tr>
<tr>
<td>49.</td>
<td>Plot of initial velocity of bleaching at 430 nm versus relative intensity of illumination. System: chlorophyll a (1.8 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-3}M) in CBE (refluxed), at rm. temp.</td>
<td>94.</td>
</tr>
<tr>
<td>50.</td>
<td>Plot of signal height versus relative intensity for change in absorbance at 430 nm, for the system: chlorophyll a (1.8 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-3}M) in CBE (refluxed), at rm. temp.</td>
<td>95.</td>
</tr>
<tr>
<td>51.</td>
<td>Absorbance changes at three wavelengths for the system: chlorophyll a (1.5 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (N_{2} purged) at -64^\circ C; to which has been added: A = plus acetic acid (0.2 ml/10 ml); B = plus 0.5% pyridine; N = no additive.</td>
<td>97.</td>
</tr>
<tr>
<td>52.</td>
<td>Plot of the change in (\Delta) absorbance (at 430 nm) with increase of acidity (log plot). System: chlorophyll a (1.7 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (refluxed) at rm. temp.</td>
<td>98.</td>
</tr>
<tr>
<td>53.</td>
<td>Absorbance changes at 430 nm (4 sec after light on). System: chlorophyll a (1.6 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-2}M) in degassed ethanol at -97^\circ C.</td>
<td>99.</td>
</tr>
<tr>
<td>54.</td>
<td>Change in initial velocity with increased added acetic acid. System: chlorophyll a (1.7 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (refluxed), at rm. temp.</td>
<td>101.</td>
</tr>
</tbody>
</table>
Figure | Page
--- | ---
55. Initial light-on rise velocities at 430 nm for the system: chlorophyll a (1.6 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-2}M) in degassed ethanol at -97°C. | 102
56. Arrhenius plot: log k (first order decay constant) versus 1/T for positive absorbance changes measured at 474 nm. System: chlorophyll a (1.7 x 10^{-2}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (N₂ purged). | 104
57. Arrhenius plot: log k (first order decay constant) versus 1/T for bleaching at 430 nm. System: chlorophyll a (1.8 x 10^{-3}M); p-benzoquinone (6.3 x 10^{-3}M) in glycerol-ethanol (degassed). | 105
58. Arrhenius plot: log k (first order decay constant) versus 1/T for bleaching at 663 nm. System: chlorophyll a (1.7 x 10^{-3}M); p-benzoquinone (6.3 x 10^{-3}M) in glycerol-ethanol (degassed). | 106
59. Arrhenius plot: log k (first order decay constant) versus 1/T for bleaching at 430 nm. System: chlorophyll a (1.8 x 10^{-3}M); p-benzoquinone (1.0 x 10^{-2}M) in degassed ethanol plus acetic acid (0.5 ml/100 ml). | 107
60. Arrhenius plot: log k (first order decay constant) versus 1/T for ESR signal decay. System: chlorophyll a (1.8 x 10^{-3}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (N₂ purged). | 108
61. Arrhenius plot: log k (first order decay constant) versus 1/T for ESR signal decay. System: chlorophyll a (1.8 x 10^{-3}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (N₂ purged) plus acetic acid (0.2 ml/10 ml). | 109
62. Arrhenius plot: log k (second order decay constant) versus 1/T for ESR signal decay. System: chlorophyll a (3.0 x 10^{-4}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (N₂ purged) plus 1% potassium acetate saturated CBE. | 110
### LIST OF ILLUSTRATIONS—Continued

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>63. <strong>Arrhenius plot</strong>: log k (first order decay constant) versus 1/T for ESR signal decay. System: chlorophyll a (1.8 x 10^{-2}M); p-benzoquinone (1.0 x 10^{-2}M) in degassed ethanol plus acetic acid (0.3 ml/100 ml).</td>
<td>111</td>
</tr>
<tr>
<td>64. <strong>Arrhenius plot</strong>: log k (first order decay constant) versus 1/T for ESR signal decay. System: chlorophyll a (4.4 x 10^{-2}M); trimethyl-p-benzoquinone (1.0 x 10^{-2}M) in CBE (refluxed).</td>
<td>112</td>
</tr>
<tr>
<td>65. <strong>Plot of Δabsorbance at 430 nm versus oxygen injected</strong> (amount of bleaching after 3 sec). System: chlorophyll a (1.3 x 10^{-2}M); p-benzoquinone (3 x 10^{-4}M) in CBE (refluxed), at rm. temp.</td>
<td>115</td>
</tr>
<tr>
<td>66. <strong>Plot of initial velocity versus oxygen injected for bleaching at 430 nm</strong>. System: chlorophyll a (1.3 x 10^{-2}M); p-benzoquinone (3 x 10^{-4}M) in CBE (refluxed).</td>
<td>116</td>
</tr>
<tr>
<td>67. <strong>Plot of steady-state Δabsorbance at 630 nm versus concentration of β-carotene and Stern-Volmer plot</strong> of the same system: chlorophyll a (1.8 x 10^{-2}M); methyl-p-benzoquinone (8.2 x 10^{-3}M) in CBE (refluxed) at rm. temp.</td>
<td>117</td>
</tr>
<tr>
<td>68. <strong>Plot of steady-state ESR signal height versus concentration of β-carotene and Stern-Volmer plot</strong> of the same system: chlorophyll a (1.8 x 10^{-2}M); methyl-p-benzoquinone (8.2 x 10^{-3}M) in CBE (refluxed), at rm. temp.</td>
<td>118</td>
</tr>
<tr>
<td>69. <strong>Plot of steady-state ESR signal height versus concentration of β-carotene and Stern-Volmer plot</strong> of the same system: chlorophyll a (1.6 x 10^{-2}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (refluxed), at rm. temp.</td>
<td>119</td>
</tr>
<tr>
<td>70. <strong>Plot of steady-state ESR signal height versus concentration of β-carotene and Stern-Volmer plot</strong> of the same system: chlorophyll a (2.5 x 10^{-5}M); p-benzoquinone (3.0 x 10^{-4}M) in CBE (refluxed) at rm. temp.</td>
<td>120</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>71.</td>
<td>Decay of bleaching at 430 nm (oscilloscope traces from flash photolysis experiments) for the system: chlorophyll a (2.5 x 10^{-5}M) in CBE (refluxed), at rm. temp.</td>
</tr>
<tr>
<td>72.</td>
<td>First order decay plots of flash excited bleaching at 430 nm for the system: chlorophyll a (2.5 x 10^{-5}M) in CBE (refluxed) at rm. temp.; with and without β-carotene (1.0 x 10^{-5}M).</td>
</tr>
<tr>
<td>73.</td>
<td>Plot of initial velocity at 460 nm versus concentration of β-carotene and Stern-Volmer plot of the same system: chlorophyll a (2.5 x 10^{-5}M); p-benzoquinone (5.0 x 10^{-4}M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>74.</td>
<td>Light-on and light-off absorbance changes as a function of time at 460 nm for the system: chlorophyll a (2.0 x 10^{-5}M); methyl-p-benzoquinone (8.2 x 10^{-3}M); β-carotene (1 x 10^{-4}M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>75.</td>
<td>Plots of steady-state signal size versus quinone redox potential for bleaching of absorbance at 670 nm and ESR signal height. System: chlorophyll a (1.9 x 10^{-5}M); quinones (5-10 x 10^{-3}M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>76.</td>
<td>Difference spectrum obtained on illumination of the system: zinc-pheophytin a (5 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>77.</td>
<td>Difference spectrum obtained on illumination of the system: cadmium-pheophytin a (ca. 1 x 10^{-5}M) (equilibrium mixture with pheophytin a); p-benzoquinone (1.0 x 10^{-2}M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>78.</td>
<td>Difference spectrum obtained on illumination of the system: bacteriochlorophyll (4 x 10^{-6}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>79.</td>
<td>Difference spectra obtained on illumination of the systems: zinc-hematoporphyrin (ca. $10^{-5}$M); p-benzoquinone ($1.0 \times 10^{-2}$M) in CBE (refluxed) at rm. temp. hematoporphyrin (1.5 $\times 10^{-4}$M); hydroquinone ($4 \times 10^{-2}$M) in pyridine-water (55:5) at $-43^\circ$C. hematoporphyrin (1.5 $\times 10^{-4}$M); p-benzoquinone ($1.0 \times 10^{-2}$M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>80.</td>
<td>Difference spectrum obtained on illumination of the system: zinc-phthalocyanine (ca. $10^{-5}$M); p-benzoquinone ($1.0 \times 10^{-2}$M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>81.</td>
<td>ESR spectrum of monoprotonated benzosemiquinone obtained on illumination of the system: pheophytin a ($1 \times 10^{-8}$M); p-benzoquinone ($1.0 \times 10^{-2}$M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>82.</td>
<td>Second order plot for the light-off decay of optical and ESR signals. System: bacteriochlorophyll ($4 \times 10^{-6}$M); p-benzoquinone ($1.0 \times 10^{-2}$M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>83.</td>
<td>Plot of the log of the absorbance change at 670 nm and ESR signal size versus quinone redox potential for the system: chlorophyll a ($1.9 \times 10^{-5}$M); quinones ($5-10 \times 10^{-3}$M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>84.</td>
<td>Plots of the log of the absorbance change and ESR signal size versus quinone redox potential for systems: zinc-pheophytin a ($5 \times 10^{-5}$M) or cadmium-pheophytin (ca. $1 \times 10^{-5}$M); quinones ($5-10 \times 10^{-3}$M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>85.</td>
<td>Plot of the log of the absorbance change and ESR signal size versus quinone redox potential for the system: zinc-hematoporphyrin (ca. $10^{-5}$M), quinones ($5-10 \times 10^{-3}$M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>86.</td>
<td>Plot of log of the absorbance change at 670 nm and ESR signal size versus quinone half-wave potential (measured in acetonitrile). System: chlorophyll a (1.9 x 10^{-5} M); quinones (5-10 x 10^{-3} M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>87.</td>
<td>Plots of the log of the absorbance change and ESR signal size versus quinone half-wave potential (measured in acetonitrile). Systems: zinc-pheophytin a (5 x 10^{-5} M) or cadmium-pheophytin (ca. 1 x 10^{-5} M); quinones (5-10 x 10^{-3} M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>88.</td>
<td>Plot of the log of the absorbance change and ESR signal size versus quinone half-wave potential (measured in acetonitrile). System: zinc-hematoporphyrin (ca. 10^{-5} M); quinones (5-10 x 10^{-3} M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>89.</td>
<td>Plots of the log of the first order light-off decay constant versus quinone redox potential. System: chlorophyll a (1.9 x 10^{-5} M); quinones (5-10 x 10^{-3} M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>90.</td>
<td>Plots of the log of the first order light-off decay constant versus quinone redox potential. Systems: zinc-pheophytin a (5 x 10^{-5} M) or cadmium-pheophytin (1 x 10^{-5} M); quinones (5-10 x 10^{-3} M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>91.</td>
<td>Plots of the log of the first order light-off decay constant versus quinone redox potential. System: zinc-hematoporphyrin (ca. 10^{-5} M); quinones (5-10 x 10^{-3} M) in CBE(refluxed) at rm. temp.</td>
</tr>
<tr>
<td>92.</td>
<td>Plot of the log of absorbance change (at 670 nm) versus the log of the first order light-off decay constant. System: chlorophyll a (1.9 x 10^{-5} M); quinones (5-10 x 10^{-3} M) in CBE (refluxed) at rm. temp.</td>
</tr>
</tbody>
</table>
LIST OF ILLUSTRATIONS—Continued

Figure | Page
--- | ---
93. Plot of the log of absorbance change (at 409 nm) versus the log of the first order light-off decay constant. System: zinc-hematoporphyrin (ca. 10^{-5} M); quinones (5-10 x 10^{-3} M) in CBE (refluxed) at rm. temp. | 154
94. Flash photolysis difference spectrum for the system: chlorophyll a (4.7 x 10^{-6} M) in CBE (refluxed) at rm. temp. \Delta absorbance was taken 0.15 millisecond after the flash. These values are for a 5 cm cuvette. | 160
95. Oscilloscope traces showing a comparison of decay kinetics for the first, sixth and thirteenth flashes at 460 nm, for the system: chlorophyll a (1.3 x 10^{-5} M) in CBE (refluxed) at rm. temp. | 161
96. Oscilloscope traces showing decay kinetics after flash photolysis of the system: chlorophyll a (1.8 x 10^{-5} M) in CBE (refluxed) at rm. temp. | 163
97. First order decay plots for oscilloscope traces shown in Fig. 96. | 164
98. Oscilloscope traces showing decay kinetics after flash photolysis of the system: chlorophyll a (7.1 x 10^{-6} M) in tert-butanol-isooctane (4:1) at rm. temp. | 165
99. First order decay plots for oscilloscope traces shown in Fig. 98. | 166
100. Oscilloscope traces showing decay kinetics after flash photolysis of the system: chlorophyll a (4.4 x 10^{-6} M) in dimethylsulfoxide at rm. temp. | 167
101. First order decay plots for oscilloscope traces shown in Fig. 100. | 168
<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>102.</td>
<td>Oscilloscope traces showing decay kinetics after flash photolysis of the system: chlorophyll a (4.5 x 10^{-6}M) in chloroform at rm. temp.</td>
</tr>
<tr>
<td>103.</td>
<td>First order decay plots for oscilloscope traces shown in Fig. 102.</td>
</tr>
<tr>
<td>104.</td>
<td>Oscilloscope traces showing decay kinetics after flash photolysis of the system: chlorophyll a (3.9 x 10^{-6}M) in carbon tetrachloride at rm. temp.</td>
</tr>
<tr>
<td>105.</td>
<td>First order decay plots for oscilloscope traces shown in Fig. 104.</td>
</tr>
<tr>
<td>106.</td>
<td>Oscilloscope traces showing decay kinetics after flash photolysis of the system: chlorophyll a (4.0 x 10^{-6}M) in ethanol at rm. temp.</td>
</tr>
<tr>
<td>107.</td>
<td>First order decay plots for oscilloscope traces shown in Fig. 106.</td>
</tr>
<tr>
<td>108.</td>
<td>Oscilloscope traces showing decay kinetics at 430 nm after flash photolysis for chlorophyll a (2.5 x 10^{-5}M) and chlorophyll a plus β-carotene in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>109.</td>
<td>First order decay plots for oscilloscope traces shown in Fig. 108.</td>
</tr>
<tr>
<td>110.</td>
<td>Oscilloscope traces and first order decay plots showing decay kinetics at 470 nm after flash photolysis for the systems: chlorophyll a (4.7 x 10^{-6}M) and chlorophyll a plus β-carotene in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>111.</td>
<td>Oscilloscope traces and first order decay plots showing decay kinetics at 660 nm after flash photolysis for the systems: chlorophyll a (2.4 x 10^{-5}M) and chlorophyll a plus β-carotene in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>Figure</td>
<td>Oscilloscope traces and first order decay plots showing decay kinetics at 520 nm after flash photolysis for the systems: chlorophyll a (4.5 x 10^-6M) and chlorophyll a plus β-carotene in degassed ethanol at rm. temp.</td>
</tr>
<tr>
<td>-------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>112.</td>
<td>180</td>
</tr>
<tr>
<td>113.</td>
<td>Oscilloscope traces and first order decay plots showing decay kinetics at 665 nm after flash photolysis for the systems: chlorophyll a (4.5 x 10^-6M) and chlorophyll a plus β-carotene in degassed ethanol at rm. temp.</td>
</tr>
<tr>
<td>114.</td>
<td>Oscilloscope traces at 2 and 5 millisec full scale sweep showing decay kinetics at 460 nm for the system: chlorophyll a (1.1 x 10^-5M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>115.</td>
<td>Oscilloscope traces at 10 and 50 millisec full scale sweep showing decay kinetics at 460 nm for the system: chlorophyll a (1.1 x 10^-5M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>116.</td>
<td>Oscilloscope traces at 100 and 1000 millisec full scale sweep showing decay kinetics at 460 nm for the system: chlorophyll a (1.1 x 10^-5M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>117.</td>
<td>Flash photolysis difference spectrum for the system: pheophytin a (5 x 10^-6M) in CBE (refluxed) at rm. temp. These Δabsorbances are for a 5 cm cuvette and were taken 0.2 millisec after the flash.</td>
</tr>
<tr>
<td>118.</td>
<td>Oscilloscope traces showing decay kinetics after flash photolysis for the system: pheophytin a (5 x 10^-6M) in CBE (refluxed) at rm. temp.</td>
</tr>
<tr>
<td>119.</td>
<td>First order decay plots (log Δabsorbance versus time) for oscilloscope traces shown in Fig. 118.</td>
</tr>
</tbody>
</table>
LIST OF ILLUSTRATIONS—Continued

Figure                                      Page

120. Oscilloscope traces and first order decay plots showing decay kinetics at 470 nm after flash photolysis for the systems: pheophytin a (5 x 10^{-6}M) and pheophytin a plus 3-carotene in CBE (refluxed) at rm. temp. ........................................... 189

121. Oscilloscope traces and first order decay plots showing decay kinetics at 410 nm after flash photolysis for the systems: pheophytin a (5 x 10^{-6}M) and pheophytin a plus 3-carotene in CBE (refluxed) at rm. temp. ........................................... 190

122. Oscilloscope traces at 470 nm (top) and 650 nm (bottom) showing decay kinetics after flash photolysis for the systems: chlorophyll a and chlorophyll a plus p-benzoquinone in CBE (refluxed) at rm. temp. ........................................... 191

123. Oscilloscope traces at 5 and 50 millisec full scale sweep times showing decay kinetics at 460 nm for the system: chlorophyll a (1.2 x 10^{-5}M), p-benzoquinone (2.0 x 10^{-4}M) in CBE (refluxed) at rm. temp. ........................................... 192

124. Oscilloscope traces at 10 and 50 sec full scale sweep times showing decay kinetics at 460 nm for the system: chlorophyll a (1.2 x 10^{-5}M), p-benzoquinone (2.0 x 10^{-4}M) in CBE (refluxed) at rm. temp. ........................................... 193

125. Oscilloscope traces showing a comparison of decay kinetics after flash photolysis at 460 nm for various concentrations of p-benzoquinone for the systems: chlorophyll a (1.2 x 10^{-5}M), p-benzoquinone (various concentrations) in CBE (refluxed) at rm. temp. ........................................... 194

126. First order decay plots for oscilloscope traces shown in Fig. 125. ........................................... 195

127. Recorder traces showing a comparison of decay kinetics at 460 nm (top) and 620 nm (bottom) after flash photolysis for various concentrations of p-benzoquinone for the systems: chlorophyll a (1.6 x 10^{-5}M); p-benzoquinone in CBE (refluxed) at rm. temp. ........................................... 196
**LIST OF ILLUSTRATIONS—Continued**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>128</td>
<td>Plots of the first order decay constants for the slowest flash decay versus benzoquinone concentration. System: chlorophyll a (1.6 x 10^{-3}M), p-benzoquinone (various concentrations) in CBE (refluxed) at rm. temp.</td>
<td>198</td>
</tr>
<tr>
<td>129</td>
<td>Oscilloscope traces showing a comparison of decay kinetics after flash photolysis at 460 nm for various quinones for the systems: chlorophyll a (1.6 x 10^{-3}M); quinones, in CBE (refluxed) at rm. temp.</td>
<td>199</td>
</tr>
<tr>
<td>130</td>
<td>First order decay plots for oscilloscope traces shown in Fig. 129 plus the first order decay plot of chlorophyll a alone in CBE at 460 nm.</td>
<td>200</td>
</tr>
<tr>
<td>131</td>
<td>Oscilloscope traces showing a comparison of decay kinetics after flash photolysis at 620 nm for various quinones for the systems: chlorophyll a (1.6 x 10^{-3}M), quinones, in CBE (refluxed) at rm. temp.</td>
<td>201</td>
</tr>
<tr>
<td>132</td>
<td>First order decay plots for oscilloscope traces shown in Fig. 131 plus the first order decay plot of chlorophyll a alone in CBE at 620 nm.</td>
<td>202</td>
</tr>
<tr>
<td>133</td>
<td>Recorder traces showing a comparison of decay kinetics at 460 nm (top) and 620 nm (bottom) after flash photolysis for various quinones for the systems: chlorophyll a (1.6 x 10^{-3}M); quinones, in CBE (refluxed) at rm. temp.</td>
<td>204</td>
</tr>
<tr>
<td>134</td>
<td>Plot of the ratio of the 460 nm first order decay constant to the 620 nm first order decay constant (k_{460}/k_{620}) versus quinone redox potential for slowest flash decay (measured by strip-chart recorder). System: chlorophyll a (1.6 x 10^{-3}M); quinone (0.93 x 10^{-4}M) in CBE (refluxed) at rm. temp.</td>
<td>205</td>
</tr>
</tbody>
</table>
135. Kinetic curves for ESR and optical experiments for the system: chlorophyll a (0.9 x 10^-5M), p-benzoquinone (1.3 x 10^-5M) in CBE (refluxed) at rm. temp. (a) Steady-state ESR signal rise and decay. (b) Steady-state optical bleaching rise and decay at 430 nm. (c) Flash optical decay at 650 nm. ... 206

136. Plots of the log of the first order flash decay constant for bleaching at 620 nm (slowest decay from strip-chart recorder) and the log of the first order steady-state ESR decay constant versus the molar ratio of benzoquinone to chlorophyll a (BQ/Chl). System: chlorophyll a, benzoquinone, in CBE (refluxed) at rm. temp. ... 207

137. Oscilloscope traces showing decay kinetics at 460 nm (top) and 620 nm (bottom) after flash photolysis for chlorophyll a (2 x 10^-5M) and chlorophyll a plus p-benzoquinone in degassed tert-butanol-isooctane (4:1) at rm. temp. ... 208

138. Oscilloscope traces showing decay kinetics at 430 nm (top), 460 nm (center) and 665 nm (bottom) after flash photolysis for chlorophyll a (4 x 10^-5M) and chlorophyll a plus p-benzoquinone in degassed ethanol at rm. temp. ... 209

139. First order decay plots of oscilloscope traces shown in Fig. 138 (center) for decay kinetics at 460 nm after flash photolysis, for chlorophyll a (4 x 10^-5M) and chlorophyll a plus p-benzoquinone in degassed ethanol at rm. temp. ... 210

140. First order decay plots of oscilloscope traces shown in Fig. 138 (bottom) for decay kinetics at 665 nm after flash photolysis, for chlorophyll a (4 x 10^-5M) and chlorophyll a plus p-benzoquinone in degassed ethanol at rm. temp. ... 211
**LIST OF ILLUSTRATIONS—Continued**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>141.</td>
<td>First order decay plots of oscilloscope traces shown in Fig. 138 (top) for decay kinetics at 430 nm after flash photolysis, for chlorophyll a (4 x 10^{-6}M) and chlorophyll a plus p-benzoquinone in degassed ethanol at rm. temp.</td>
<td>213</td>
</tr>
<tr>
<td>142.</td>
<td>Oscilloscope traces showing decay kinetics at 410 nm (top) and 460 nm (bottom) after flash photolysis for pheophytin a (5 x 10^{-6}M) and pheophytin a plus p-benzoquinone in CBE (refluxed) at rm. temp.</td>
<td>214</td>
</tr>
</tbody>
</table>
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. First order light-off and light-on constants for various wavelengths and ESR signal. System: chlorophyll a (2 x 10^{-5}M); 2,5-dichloro-p-benzoquinone (1.1 x 10^{-3}M) in CBE (refluxed) at rm. temp.</td>
<td>76</td>
</tr>
<tr>
<td>2. First order light-off and light-on constants for various wavelengths and ESR signal. System: chlorophyll a (2 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (refluxed), at rm. temp.</td>
<td>77</td>
</tr>
<tr>
<td>3. First order light-off and light-on constants for various wavelengths and ESR signal. System: chlorophyll a (2 x 10^{-5}M); methyl-p-benzoquinone (0.9 x 10^{-2}M) in CBE (refluxed), at rm. temp.</td>
<td>78</td>
</tr>
<tr>
<td>4. First order light-off and light-on constants for various wavelengths and ESR signal. System: chlorophyll a (2 x 10^{-5}M); 2,5-dimethylbenzoquinone (0.6 x 10^{-2}M) in CBE (refluxed), at rm. temp.</td>
<td>79</td>
</tr>
<tr>
<td>5. First order light-off decay constants for various wavelengths and ESR signal. System: chlorophyll a (2 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE, at rm. temp.</td>
<td>83</td>
</tr>
<tr>
<td>6. ESR and optical decay constants for various ratios of p-benzoquinone to chlorophyll a for the system: chlorophyll a; p-benzoquinone in CBE (refluxed), at rm. temp.</td>
<td>87</td>
</tr>
<tr>
<td>7. First order light-off decay constants as affected by added acid and base. System: chlorophyll a (1.5 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (N_2 purged) at -64°C.</td>
<td>100</td>
</tr>
<tr>
<td>8. Activation energies for chlorophyll-quinone systems (from Arrhenius plots)</td>
<td>113</td>
</tr>
<tr>
<td>Table</td>
<td>Page</td>
</tr>
<tr>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>9. Calculated $k_3$'s from optical and ESR experiments at room temp. in CBE (refluxed).</td>
<td>125</td>
</tr>
<tr>
<td>10. Initial velocities of light-on rise signal for four quinones at three wavelengths. System: chlorophyll a (1.9 x $10^{-3}$M); quinones (5-10 x $10^{-3}$M) in CBE (refluxed) at rm. temp.</td>
<td>155</td>
</tr>
<tr>
<td>11. First order light-off decay constants and percent $\Delta$absorbance for steady-state bleaching for several metal-porphyrins and quinones. Systems: metal-porphyrin (about $10^{-3}$M), quinone ($10^{-2}$M) in CBE (refluxed) at rm. temp.</td>
<td>225</td>
</tr>
</tbody>
</table>
ABSTRACT

ESR spectroscopy of chlorophyll-quinone mixtures in degassed alcohol solutions shows only the formation of semiquinone anion radical on illumination. Other porphyrins, both chelated and non-chelated, and hydroquinone can serve in the above reaction as well. No ESR signal due to chlorophyll is observed. Similarly, an optical spectrum of the illuminated system shows only the semiquinone absorbance band. Decay of the signals is second order.

Optical changes due to chlorophyll can be elicited by lowering the temperature of the alcohol to a point of high viscosity. For these optical changes, hydroquinone cannot substitute for quinone.

Similar results are achieved by using a degassed viscous mixture of the alcohols: cyclohexanol, tert-butanol, and ethanol (CBE). Difference spectra show bleaching of the chlorophyll bands and increased absorbance in the intermediate region (460-580 nm). Decay kinetics are first order, while the rise is complicated (possibly biphasic). ESR signals have no hyperfine structure and also decay by first order kinetics, at a faster rate than optical changes. The ESR signal reaches a steady state more rapidly than optical signals, without biphasic kinetics. From these effects, we
postulate that two species are generated, one paramagnetic, and one not.

Acidity increases the amount of bleaching in CBE, basicity decreases it and strong basicity causes bleaching to completely disappear with only the semiquinone anion being observed. Activation energies for chlorophyll $a$ and benzoquinone in CBE are $10^{-14}$ kcal/mole. Lower potential quinones give lower activation energies. The rate constant for quenching the triplet state of chlorophyll $a$ by $\beta$-carotene in CBE $\geq 7.5 \pm 0.5 \times 10^8$ (M sec)$^{-1}$. The bimolecular rate constant for formation of the photoproduct with benzoquinone was calculated to be $\geq 7 \times 10^8$ (M sec)$^{-1}$.

Redox potential of the quinone affects both the magnitude of chlorophyll absorbance changes and rate of decay. The higher the potential, the larger the changes and slower the decay. The log of $\Delta$ absorbance versus redox potential of quinone plots as a straight line. From such plots, we have calculated photopotentials ( $+0.29$ v (NHE) for chlorophyll $a$). Other porphyrin systems show similar effects only if they are chelated with a group II metal, like Mg$^{2+}$.

Flash photolysis of chlorophyll $a$ alone in CBE gives a difference spectrum similar to those obtained by steady illumination of chlorophyll $a$ - quinone mixtures in CBE. Decay kinetics in CBE and dimethylsulfoxide are faster
at the Soret band than at 460-580 nm and red band regions. This difference is not obtained in less viscous solvents (CHCl$_3$, CCl$_4$, tert-butanol, ethanol), implying that two or more species are observed in CBE and DMSO. Also, decay rates follow no simple kinetic order.

\[\beta\]-carotene in CBE quenches bleaching at 430 and 660 nm but not absorbance at 470 nm. So bleaching must be primarily due to triplet formation while the 470 nm absorbance is due to a product of the triplet state. This effect is not observed in ethanol.

Adding quinone results in slowly-decaying species being generated by the flash. Three components can be distinguished: the first ($t_\frac{1}{2} \sim 0.2$ msec) corresponds to the triplet state; the second ($t_\frac{1}{2} = 5-10$ msec) is quinone concentration and species independent; the third ($t_\frac{1}{2} = $ several seconds) is dependent on quinone concentration and species (rate is faster for higher concentrations and lower potential quinones). The ESR decay is approximately equal to the third component flash decay when chlorophyll concentration equals quinone. With excess quinone, the flash decay becomes faster, and the ESR decreases slightly. None of these slow-decaying species are produced when quinone is added to chlorophyll $a$ in ethanol or tert-butanol, or pheophytin in CBE.
Mechanisms to account for all of these phenomena are put forward, which involve several species of chlorophyll-quinone complexes, free radicals and solvent interactions.
CHAPTER 1

INTRODUCTION

The process of photosynthesis begins with the absorption of a quantum of light by plant pigments. Chlorophyll is the main photosynthetic pigment. In green plant photosynthesis an overall oxidation-reduction takes place: hydrogen derived ultimately from water is utilized for the reduction of CO$_2$ to carbohydrates and water is oxidized to molecular oxygen. Since chlorophyll is a component in the chain of steps resulting in hydrogen transfer from H$_2$O to CO$_2$, it would be reasonable to investigate the possible photochemical oxidation or reduction of this pigment. Indeed, studies of isolated chlorophyll in suitable media and with electron acceptors and donors do show evidence of reversible oxidation-reduction (Seely 1966).

The purpose of this dissertation is to report on investigations using isolated chlorophyll and chlorophyll analogs in one electron photo-oxidation and reduction reactions with quinones and hydroquinones. It was hoped that a study of the intermediate states in these reactions might provide insight into the functioning of chlorophyll as an absorber and transducer of light energy in living photosynthetic systems.
Chlorophyll is the green dihydroporphyrin (chlorin) pigment found ubiquitously in green plants and algae. The tetrapyrrolic nitrogens of the chlorophyll molecule are chelated to a central magnesium atom (Willstätter and Stoll 1913). However the nitrogen-magnesium bonds are more nearly ionic than covalent (Seely 1966) and therefore the metal ion is quite easily lost to the solvent under mild acidic conditions. Four types of chlorophyll, designated a, b, c and d, have been isolated from green plants and algae (Holt 1965). Chlorophyll a and b are found in all the higher plants, usually in a ratio of about 3 to 1. The only difference between these two compounds is that whereas chlorophyll a has a methyl group at position 3 (see structure, Fig. 1), chlorophyll b has a formyl group. The electron attracting property of the formyl group on the pyrrolenine ring (II) gives the visible spectrum of chlorophyll b a hypsochromic shift (Seely 1966). The visible spectrum of chlorophyll a is shown in Figs. 2 and 3; that of chlorophyll b is shown in Fig. 4. Chlorophyll d is also dissimilar to chlorophyll a only in one sidechain; a formyl group replaces the vinyl group at position 2 (Holt and Morley 1959). Chlorophyll d is found in red algae along with chlorophyll a (Holt 1965).
Figure 1. Chlorophylls a and b
Figure 2. Visible spectrum of chlorophyll a in diethyl ether.
Figure 3. Visible spectrum of chlorophyll $a$ in CBE (cyclohexanol-tert-butanol-ethanol 675:200:125).
Figure 4. Visible spectrum of chlorophyll b in diethyl ether.
The phytol tail which is esterified onto the 7-propionic acid side chain makes up about one-third of the weight of these chlorophylls (Willstätter and Stoll 1913). It is responsible for the surface activity of chlorophyll and the extreme difficulty of its forming crystals. It is thought that the association of chlorophyll with lipoprotein in the chloroplast is through the phytol tail (Seely 1966).

There are three asymmetric carbons: 7, 8 and 10. However carbon atom 10 does not function as a center of asymmetry due to the ease in which the carbonyl group in position 9 is enolized (Rothemund 1949). Chlorophylls \( \text{a}^* \) and \( \text{b}^* \), which are identified by their bands on a chromatography column at slightly different positions than those of the natural pigments, are thought to be C-10 epimers.

Chlorophyll \( \text{a} \) is a widely distributed chlorophyll pigment found together with chlorophyll \( \text{a} \) among marine algae (diatoms, dinoflagellates and all brown algae) (Strain and Svec 1966). It is found as a one-to-one mixture of two forms which vary only in the side chain at position 4 (see structure Fig. 5) (Dougherty et al. 1966).

While chlorophyll \( \text{a} \) is the major photosynthetic pigment among green plants and algae, bacteriochlorophyll, a tetrahydroporphyrin, plays this role among the photosynthetic bacteria (see structure Fig. 6 and visible spectrum
Figure 5. Chlorophyll a
Figs. 7 and 8). In many of these organisms, except for the carotenoids, it is the only photosynthetic pigment present. Green sulfur bacteria such as *Chlorobium* and *Chloropseudomonas* contain a dihydroporphyrin photosynthetic pigment called chlorobium chlorophyll. Unlike chlorophyll a or b, chlorobium chlorophyll does not represent a unique compound. Side chains at positions 4 and 5 (Fig. 9) can have a few different alkyl substituents and the $\delta$-bridge carbon can have either a hydrogen, methyl or ethyl group attached. Those chlorobium chlorophylls which have hydrogen attached to the $\delta$-bridge carbon have their red band absorbing at 650 nm, while those with methyl or ethyl absorb at 660 nm (Holt *et al.* 1963, Hughes and Holt 1962).

Finally, we should indicate the structure and visible spectra of a few porphyrin and porphyrin-like pigments which were also used in the experiments to be described below as analogs of chlorophyll: protoporphyrin IX (structure Fig. 10), hematoporphyrin IX (structure Fig. 11, visible spectrum Fig. 12), zinc-hematoporphyrin IX (visible spectrum Fig. 13), zinc-phthalocyanine (structure Fig. 14, visible spectrum Fig. 15), and the visible spectra of pheophytin a, zinc-pheophytin a and cadmium-pheophytin a and b in equilibrium with pheophytin (Figs. 16, 17 and 18).
Figure 6. Bacteriochlorophyll
Figure 7. Visible spectrum of bacteriochlorophyll in diethyl ether.
Figure 8. Visible spectrum of bacteriochlorophyll in CBE.
Figure 9. Chlorobium Chlorophyll
Figure 10. Protoporphyrin IX
Figure 11. Hematoporphylin IX
Figure 12. Visible spectrum of hematoporphyrin IX in pyridine-water (55:5).
Figure 13. Visible spectrum of zinc-hematoporphyrin in CBE.
Figure 14. Zinc-phthalocyanine
Figure 15. Visible spectrum of zinc-phthaloacyanine in ether.
Figure 16. Visible spectrum of pheophytin a in CBE.
Figure 17. Visible spectrum of zinc-pheophytin a in CBE.
Figure 18. Visible spectrum of cadmium-pheophytin a and b (in equilibrium with free pheophytin) in CBE.
Role of Chlorophyll in Green Plant Photosynthesis

The bulk of the chlorophyll in the chloroplast serves to harvest light quanta (Emerson and Arnold 1932, Gaffron and Wohl 1936, Clayton 1968), converting them to electronic excitation energy. Because of the close proximity of the chlorophyll molecules in the chloroplast (Thomas, Minnaert and Elberts 1956), this excitation energy can migrate from chlorophyll to chlorophyll. Three possible mechanisms for migration of excitation energy among these molecules are summarized as follows:
(1) Resonance transfer (Clayton 1966).
(2) As an "exciton" (via an interaction between the transition dipole on one molecule and an induced dipole on its neighbor).
(3) As positive holes and electrons moving independently of each other (Calvin and Sogo 1957). In this case one visualizes the chlorophyll molecules making up a semiconductor with the overlap of the excited states forming a "conduction band" where charge carriers may move freely (Lavorel 1966, pp. 155-160).

Ultimately a chlorophyll molecule in a special environment is excited (Kok 1956, 1961). This chlorophyll is believed to be in close proximity to an electron acceptor (Clayton 1967). The reduction of the electron acceptor by
the excited chlorophyll begins an electron transfer process which ultimately ends in the reduction of pyridine nucleotide (Vernon and Ke 1966) and the formation of ATP. Reduced pyridine nucleotide and ATP are then utilized in the dark reactions which fix CO₂ to form carbohydrate material (Arnon 1968).

In the scheme of green plant photosynthesis which is accepted by most workers in the field, the excitation of two separate chlorophyll systems are involved (Avron 1969). These two systems, called system I and system II, are coupled together by an electron transport system consisting primarily of membrane-bound components having electron carriers similar to those of the mitochondrial respiration system. Light absorbed by the system II chlorophyll raises an electron from the potential of water at +0.8 volt to around 0.0 volt. The electron then flows through the electron transport system to system I at +0.4 volt. The production of ATP is coupled to this flow. Light absorbed by system I chlorophyll lifts the electron from the potential of cytochrome f at +0.4 volt to that of ferredoxin at -0.4 volt (A stronger reductant, "X", of about -0.6 volt is thought to be produced first (Zweig and Avron 1965; Kok, Rurainski and Owens 1965; Black 1966)). Then, via reduced pyridine nucleotide, the electron enters the carbon reduction cycle.
Electronic Excited States of Chlorophyll

Light absorbed in the visible region of the electromagnetic spectrum by chlorophyll, and porphyrins in general, is associated with $\pi$-electronic transitions to singlet excited states. The low energy (visible and near UV region) of these $\pi \rightarrow \pi^*$ transitions is a consequence of the extensive delocalization of the $\pi$-electrons in the ring system (Falk 1964). These $\pi$-electrons are displaced in the plane of the ring (Goedheer 1966) toward the periphery on excitation. The source of the $\pi$-electrons are different for the two main bands of chlorophyll. The Soret band is identified as a $\pi \rightarrow \pi^*$ transition of electrons located on the carbon atoms of the pyrrole which move toward the periphery of the ring. The red band is a $\pi \rightarrow \pi^*$ transition in which the electrons flow from pyrrole nitrogens to peripheral carbon atoms (Maggiora and Ingraham 1967). The satellite bands which are found adjacent to both main bands at shorter wavelengths result from different processes. In the case of the Soret band, these are probably due to transitions to higher energy vibrational sublevels. In the case of the lowest energy transition, these bands are generated by electronic excitations occurring parallel and perpendicular to the long axis of the chlorophyll molecule (i.e., the axis which traverses the nitrogen atoms of ring II and IV. See Fig. 1.) (Clayton 1965).
In dilute solutions of chlorophyll, the lowest singlet excited state can decay via emission of a light quantum (fluorescence), by intersystem crossing to the metastable triplet state or by dissipation as heat or chemical interaction with the surrounding medium. In polar solvents, the first two are the only important mechanisms. It has been shown that the sum of the quantum yields for fluorescence and triplet formation nearly approaches unity (Bowers and Porter 1967).

Only fluorescence corresponding to the lowest electronic singlet state of chlorophyll (i.e., the red band) is obtained in solutions of chlorophyll. The quantum efficiency and spectral distribution of fluorescence are independent of whether the light one uses to excite the chlorophyll is red or blue. This does not hold for wavelengths on the low energy side of the red band (Goedheer 1966). A non-radiative transition transfers excitation energy from the higher singlet states (Soret band) to the lower electronic state (red band) (Livingston 1960).

The quantum yield of fluorescence and triplet formation of chlorophyll \( a \) in a polar solvent (ether) are 0.32 and 0.64 respectively. The quantum yields for chlorophyll \( b \) show a lower yield for fluorescence and a correspondingly higher yield for triplet formation: 0.12 and 0.88
respectively (Bowers and Porter 1967). The fluorescence lifetime is of the order of $10^{-9}$ sec. (Brody and Rabinowitch 1957, Butler and Norris 1963).

The spectral technique of flash photolysis is required to observe the triplet absorption spectrum of chlorophyll, since the decay time (in ether) is only about 500 $\mu$sec (Bowers and Porter 1967). Oxygen must be rigorously eliminated from the solvent because of its efficient quenching of the triplet state. The absorption spectrum thus obtained shows a broad band with a maximum at about 460 nm and a weaker band at about 520 nm and gradually tails off into the red (Linschitz and Sarkanen 1958).

Since the triplet state is a paramagnetic state, one would expect to see electron spin resonance signals associated with its formation. Rikhireva et al. (1964) observed two ESR signals on illuminating frozen alcohol solutions of chlorophyll a. They associated the triplet state of chlorophyll with a signal at about 1500 gauss and a radical state with a signal in the 3000 gauss range. Lhoste (1968) obtained ESR signals in the above regions from the illumination of chlorophyll b in degassed ethanol at 80°K. The strong single line at about 1650 gauss and the smaller one at 3300 gauss was identified with the $\Delta m = \pm 2$ triplet sublevel transition. The complex spectrum
from about 2900 to 3600 gauss was identified as an anisotropic \( \Delta m = \pm 1 \) magnetic dipole transition. The triplet state of chlorophyll is not observed in green plants under normal conditions. Only when the bulk of the chlorophyll is decreased to about 1\% of its original amount is the triplet state observed (Witt, Müller and Rumberg 1963).
The Photo-oxidation and Photo-reduction of Chlorophyll
as a Model of Photosynthetic Activity

In the utilization of excited state energy in the chloroplast, an electron from excited chlorophyll is thought to be fully removed and transferred to another molecule, starting a chain of oxidation-reduction steps. The oxidized chlorophyll, a free radical, decays by receiving electrons from the oxygen evolution system (system II). The rapidly decaying ESR signal obtained when chloroplast material is illuminated in an ESR cavity is thought to be due to this oxidized chlorophyll free radical, called "P700" or the system I photo-reaction center (Vernon, Ke and Shaw 1967; Vernon, Shaw and Ke 1966). Many model systems have been investigated with chlorophyll in the presence of electron acceptors or donors or with both electron acceptors and donors present. These systems have demonstrated that one-electron transfer reactions occur mediated by light-excited chlorophyll. Depending on several factors, an electron can be transferred to or from the chlorophyll molecule upon light excitation.

Chlorophyll Photo-reduction

Ascorbic acid in degassed pyridine (with a small amount of water) was shown to photoreduce chlorophyll in the well-known "Krasnovskii reaction" (Krasnovskii 1948).
Here, two one-electron reduction steps occur, the first of which forming a fast decaying reduced chlorophyll free radical, ChlH• (Livingston and Pugh 1960, Livingston and McCartin 1963, Zieger and Witt 1961), and the second forming the more stable dihydrochlorophyll, ChlH2, the "red product" with absorption maximum at 525 nm. These two reactions occur only in basic media and with a limited number of electron donors of sufficient reducing potential.

The reductant, hydroquinone, in either neutral ethanol or basic pyridine-water mixtures has been shown to undergo a one-electron oxidation sensitized by the chlorophyll triplet state (Banerjee and Tollin 1966, White and Tollin 1967, Cho and Tollin 1968, Mukherjee, Cho and Tollin 1969). No ChlH2 is formed, however, possibly due to a fast recombination between chlorophyll radical and quinone radical (Banerjee and Tollin 1966) or to quick oxidation of the dihydrochlorophyll by quinone produced from disproportionation of the semiquinone radical. In support of this latter suggestion, the Krasnovskii red intermediate is not produced in the presence of quinone (Krasnovskii and Gavrilova 1951).

Quinlan and Fujimori (1967) measured the change in pH on illuminating a degassed solution of chlorophyll and hydroquinone in methanol. They obtained a decrease in pH on illumination (from about 8.40 to 8.33). This pH change
was completely reversible. From this they postulated that protons were being ejected into the surrounding medium during a light-induced one-electron transfer between chlorophyll and hydroquinone. Simpson, Freeman and Reucroft (1970) measured the photovoltage of illuminated chlorophyll-hydroquinone solutions in ethanol at different pH's. They obtained positive photovoltages above pH 7 and negative photovoltages at pH 7 and below. The mechanism for this according to them involves proton ejection by chlorophyll. In the acid solution, the proton is the electrode-active species while in the basic solution, the proton is not mobile so that the negative ions (ChlH⁻ and Q⁻) are those which become the electrode-active species.

Chlorophyll Photo-oxidation

Porret and Rabinowitch (1937) first observed that chlorophyll solutions in methanol, purged of oxygen, underwent a reversible bleaching of about one percent upon illumination with red light. The bleaching was found to decay with second order kinetics and to have a decay time anywhere from one to 100 seconds, depending on impurities in the solvent (Livingston 1941).

Adding a small amount of formic acid increases the steady-state bleaching from one percent to as much as 10 to 30 percent. Also, 10⁻⁴M oxalic acid gives a threefold
enhancement of the steady-state bleaching, a tenfold increase in the halflife and changes the kinetic order of the back reaction from second to first order (McBrady and Livingston 1948).

Other additives such as methyl red and iodine have an enhancement effect on bleaching. Iodine has a particularly strong effect, increasing the steady-state bleaching from 0.2 to 26%, the halflife from 0.5 to 20 sec and changing the back reaction from second to first order (Rabinowitch 1956).

An oxygen concentration as low as $10^{-6}$ M suppresses the bleaching completely, suggesting triplet state involvement. Likewise ferrous chloride also inhibits the bleaching of chlorophyll. However if an equimolar amount of ferric and ferrous chloride is added to the methanol, no inhibition of the bleaching is experienced. This led Rabinowitch to propose that the chlorophyll was undergoing photo-oxidation with either the solvent or a solvent impurity (Rabinowitch 1945).

Livingston and Ryan (1953), using chlorophyll illuminated in degassed methanol, roughly measured the spectrum of the bleached chlorophyll. They estimated the absorption coefficients of the photoproduct by assuming that its coefficient was zero at the wavelength where bleaching was maximum. This assumption turns out not to be true, so that
each value must be corrected by a small constant amount.

Stockman (1961) investigated the bleaching of chlorophyll in solvents of different viscosity (from ethyl acetate to castor oil) and found that the magnitude of bleaching and the rate of the back reaction were independent of viscosity. Further, in castor oil, no temperature coefficient was found over a 54 degree (centigrade) range.

Linschitz and Rennert (1952) illuminated oxygen-free chlorophyll solutions in ether-isopentane-ethanol (EPA) (8:3:5) at $-190^\circ$C. Bleaching occurred in the Soret and red band regions and absorption increased in the intermediate region (480-590 nm) and the far red (700-740 nm). An enhancement of bleaching took place on adding $10^{-2}$ to $10^{-3}$M quinone or imine. In the presence of quinone, the bleached state was stable in the dark until the solvent was melted. Evstigneev, Gavrilova and Krasnovskii (1950) found, however, that quinone caused no steady-state photobleaching of chlorophyll in ethanol at room temperature.

Tollin and Green (1962) examined illuminated solutions of chlorophyll in degassed EPA in the presence of quinone at low temperatures by ESR spectroscopy. At $-45^\circ$C they obtained an ESR signal due to the semiquinone free radical. No signal derived from chlorophyll itself was produced. The maximum size of the five-line signal for benzosemiquinone occurred between $-80^\circ$ and $-100^\circ$C. At $-120^\circ$C
they obtained a mixture of two signals, the five-line semiquinone signal and a broad signal. The ratio of the broad signal to the semiquinone signal increased on decreasing temperature. Below -130°C only the broad signal was obtained. Signals in ethanol behaved the same way, except that the broad signal was detected at a higher temperature (-95°C). The authors interpreted this latter signal as evidence for a chlorophyll-quinone radical complex. The photochemistry occurring in these systems is completely reversible in the dark, the decay of the semiquinone radical resulting from disproportionation (Mukherjee, Cho and Tollin 1969). Radical formation has been shown to involve the chlorophyll triplet state (White and Tollin 1967).

The present dissertation represents an extension of the optical and ESR investigations of the chlorophyll-quinone and chlorophyll-hydroquinone systems in alcoholic solutions. The main goal of this work was to obtain further information which would help to clarify the way in which chlorophyll participates in these photoredox reactions.
CHAPTER 2

CHEMICAL PREPARATIONS AND INSTRUMENTATION

Chlorophyll a was isolated six times during the course of these experiments. In all cases spinach was the source of the chlorophyll. On one occasion, it was obtained from a package of frozen spinach (Bird's Eye), but all other times it was obtained fresh from a local produce wholesaler.

Chemical Preparations

Extraction of Chlorophyll a

The method of extraction used was that of Strain and Svec (1966) with the following minor variations:

1. The principal petroleum ether pigment extract was washed with aqueous methanol about ten times to remove as much xanthophyll as possible. Afterwards, it was swirled very gently with saline water three times to remove methanol.

2. To prevent precipitation of the pigments from the petroleum ether, a little reagent grade acetone was added instead of diethyl ether.

3. Two powdered sugar chromatographic separations were performed to purify the pigment. The first was to eliminate
the remaining carotenoids and the second to separate the chlorophylls. The chromatography column, measuring 4.5 cm in diameter and having a height of about 50 cm, was packed with approximately 1-1/2 lbs of C & H confectioners sugar. The sugar, obtained from a freshly opened box, was sifted and used without any further treatment.

4. Chlorophyll was removed from the column by scooping out sections containing the bands and extracting the pigment with diethyl ether. The diethyl ether solution was washed with water to remove sugar and evaporated to dryness using a water aspirator. The dry chlorophyll was stored in a glass stoppered bottle under a nitrogen atmosphere and kept in a freezer.

Metal Chelates of Pheophytin, Hematoporphyrin and Phthalo-cyanine

Zinc-pheophytin, cadmium-pheophytin, zinc-hematoporphyrin and zinc-phthalocyanine were prepared by heating zinc or cadmium acetate with pheophytin, hematoporphyrin or phthalocyanine in various solvents such as acetone, pyridine or acetic acid. All of these metal chelates were purified by paper chromatography. Cadmium-pheophytin gave a chlorophyll-like spectrum in diethyl ether but when dissolved in alcoholic solvents, it equilibrated to a mixture of about 30% cadmium-pheophytin and 70% pheophytin. Phthalocyanine was found to be very
insoluble in almost all solvents. However zinc-phthalocyanine was readily soluble in alcohol, giving an azure colored solution. Mercury, cobalt and nickel chelates of pheophytin were also prepared in the same way.

Extraction of Bacteriochlorophyll

*Rhodospirillum rubrum* cells were collected by centrifugation and bacteriochlorophyll was extracted with a methanol-diethyl ether-petroleum ether solution as outlined by Strain and Svec (1966). Separation from carotenoids was accomplished by paper chromatography using petroleum ether containing 0.5% n-propanol as solvent.

Reagents

The following reagents are those used in this dissertation. Their source and purification, if any, are listed below:

Porphyrrins:

Pheophytin *a + b* (puriss.), Fluka AG, Buchs SG #77420.
Protoporphyrin IX (C grade), Cal Biochem #5395.
Hematoporphyrin•2HCl, Cal Biochem #3731, chromatographed.
Hematoporphyrin, free base, Nutritional Biochemicals Corp. #4482, chromatographed.
Phthalocyanine, Eastman #9495.
Quinones:

$p$-Benzoquinone (pract.), Eastman P220, sublimed.
p-Hydroquinone, Eastman #356.
Methyl-$p$-benzoquinone (pract.), Eastman P3520, sublimed.
$2,5$-Dimethyl-$p$-benzoquinone, Eastman #3710, sublimed.
Trimethyl-$p$-benzoquinone, K & K Labs., Inc. #19543.
$1,4$-Naphthoquinone (pract.), Eastman P1704, sublimed.
$2,5$-Dichloro-$p$-benzoquinone, Aldrich.

Solvents:

Ethyl alcohol (absolute), U.S.I.
Cyclohexanol, Matheson Coleman & Bell #2758, distilled.
tert-Butyl alcohol, Baker Analyzed Reagent #9056, distilled.
Glycerol (Spectroquality Reagent), Matheson Coleman & Bell #SG-5089.
tert-Butyl acetate, Matheson Coleman & Bell #9225, distilled over CaCl$_2$.
Pyridine (A.R.), Mallinckrodt #7180.
Dimethyl sulfoxide, Matheson Coleman & Bell #7963.

Others:

$\beta$-Carotene, Matheson Coleman & Bell #9192.
$\beta$-Carotene (Ampoule, 80-90% $\beta$-isomer), Sigma Chemical Co.
Degassing Techniques

Three methods of solution degassing were employed in these experiments. For non-viscous solvents the freeze-thaw method was used (Tollin and Green 1962). For viscous solvents consisting of a mixture of alcohols this method was not used because it had the effect of distilling off the lower boiling alcohol, thus changing the ratio of the components.

In the earliest experiments, such samples were purged with nitrogen gas. A fine capillary which was drawn out from glass tubing delivered a stream of nitrogen bubbles into the cuvette sample for 3/4 hour. Dye-Ox dry nitrogen gas was passed over hot copper turnings in a deoxygenation furnace (Sargent #S-36517). The copper was regenerated after every experiment by passing a stream of hydrogen gas over the turnings while hot. A small amount of solvent did evaporate during purging. Therefore purging time for all experiments was kept the same.

For most of the experimental work, degassing by purging with nitrogen gas was abandoned in favor of the reflux method, which could be applied more reliably. It was found that sample kinetic properties varied considerably depending on the incidentals of purging, i.e., length of time, flow rate of the purging gas and size of the
vessel being used. This last item was especially important in connection with comparing kinetic parameters between optical and ESR signals.

Refluxing of the solvent was done in a two-liter round bottom flask with a heating mantle. The flask was fitted with two refluxing columns which had self-contained water jackets. It was half filled with solvent and washed graphite boiling chips were added. The Variac was adjusted so that only a very mild boiling took place. This was allowed to proceed for 24 hours. Refluxing was stopped by immersing the flask in ice while at the same time a gentle stream of nitrogen gas was circulated in the flask through a two-hole stopper. Positive pressure of the nitrogen gas kept air from being sucked in while the solvent cooled. Through the hole in the stopper from which nitrogen gas escaped, a glass tube was inserted. This tube was used for transferring the solvent. When the solvent was cooled, an amber surgical tube was connected to the end of the glass tube outside of the flask. The end of this tube was placed in a second vessel to which solvent was to be transferred. Transferring was accomplished by lowering the glass tube under the surface of the solvent. The pressure of the nitrogen gas forced solvent through the tube into the vessel.
Instrumentation

Optical Spectroscopy during Steady Illumination

The sample in a one cm square cuvette was placed in a copper cryostat (both shown in Fig. 19). This cryostat, a modification of that designed by Jones and Willard (1956), was made so that the optical density of the sample could be measured while irradiating at right angles. A coolant (in these experiments mainly dry ice-ethanol) was placed in the chamber surrounding the upper part of the cuvette shaft. The heavy copper walls of the lower part of the shaft efficiently conducted heat away from the sample. A flow of dry nitrogen gas was blown up from the bottom of the cryostat to avoid condensation of water on the windows. With this technique, condensation was not a problem except on the most humid days. To some extent, the temperature of the sample could be regulated by adjusting the nitrogen gas flow. Temperature was measured by means of a copper-constantan thermocouple incorporated inside the cuvette.

The cryostat and cuvette were placed in the sample compartment of an optical spectrophotometer. This apparatus employed a modulated light beam (chopper) and phase sensitive detection to filter out noise, and was able to detect optical density changes of the order of $10^{-3}$ units. This was originally designed as a flash spectrophotometer.
Figure 19. Cryostat and sample cuvette for measuring optical density.
(Green and Tollin 1967), but in these experiments flash illumination was not used and the xenon flash tubes were removed from the sample compartment.

A hole was drilled into the side of the sample compartment aligned with the window of the cryostat and perpendicular to the two measuring windows. Light from a Kodak Sun Gun (Model 2) with an iodine-quartz lamp was focused into this hole by two condensing lenses. This light was filtered by a 1-1/2 inch thick water filter, three 1/4 inch infrared absorbing glass plates and a Corning glass filter. The illumination could be interrupted by a manual shutter (shutter time was estimated at 0.1 sec). A schematic drawing of this apparatus is shown in Fig. 20.

A YSI Model 65 Radiometer was used to measure the light intensity reaching the sample cuvette upon illumination. The Radiometer probe was placed in the sample compartment and aligned to register the greatest amount of light intensity. The position of the light source was then readjusted for maximum intensity. With a Corning 2-61 red filter, an intensity of $8.4 \times 10^4$ ergs/cm$^2$·sec was obtained.

Optical density measurements were made one wavelength at a time. A blocked interference filter was placed in front of the sample photomultiplier. A wire screen of known optical density was also in the light path of the sample. For each setting of the apparatus in which a run
Figure 20. Schematic of apparatus for steady illumination of sample during optical density measurements.
was made, the screen was moved in and out, creating a deflection of the recorder. This deflection was used to calculate the optical density changes occurring at each wavelength. Rise and decay kinetics were monitored by a Sanborn Type 151 recorder.

ESR Spectroscopy during Steady Illumination

ESR spectra were obtained with a Varian 100 KC modulation spectrometer, equipped with a V-FR2200 Fieldial unit and a TE-102 cavity. Light from a Kodak Sun Gun was focussed into the cavity through a water filter, three infrared absorbing glass plates, condensing lenses, manual shutter and Corning glass filter, in a set up similar to the one mentioned previously. In addition, a small plano-convex lens with a focal length of about two inches was placed right in front of the cavity to focus the light sharply into the slits of the cavity. These slits allowed approximately 50% of the light incident upon them to enter.

Two chart recorders were used with this ESR machine: A Leeds & Northrup Type G Speedomax recorder which had good sensitivity but a one second response time and maximum chart speed of only about 5 mm/sec, and a Sanborn Type 151 which had less sensitivity but could be used for faster kinetics (response time: 0.01 sec, maximum chart speed: 100 mm/sec).
Samples were run in a quartz capillary tube designed for freeze-thaw degassing (Fig. 21). These could be run at low temperatures by passing a stream of nitrogen gas through the Varian Low Temperature Probe which had been cooled by passing through coils submerged in a dewar of liquid nitrogen. Temperature was adjusted by the flow of nitrogen gas. A copper-constantan thermocouple incorporated in the quartz tube was used to measure temperatures.

Flash Spectroscopy

A schematic for the flash photolysis apparatus is shown in Fig. 22 (Raman and Tollin 1970). This apparatus was designed for two flash lamps, but only one was used in these experiments. A Corning 3-66 orange filter was mounted between the flash lamp and the sample cell. The lamp was fired upon charging a 7.5 MFD capacitor to about 6000 volts. In the red region of the spectrum, the flash artifact lasted between 100 and 150 \( \mu \text{sec} \), so that most kinetic data were taken after this time. In many cases, especially at shorter wavelengths, the flash artifact was small enough so that kinetics in the 50 to 100 \( \mu \text{sec} \) range could also be measured.

The sample compartment was surrounded on two sides by curved polished metal reflectors and cooled by forced air. A 5 cm quartz Cary cell contained the sample being
Figure 21. ESR tube for degassed samples.
Figure 22. Schematic of flash apparatus.
studied. Degassing by freeze-thaw took place in a separate 20 ml round bottom flask with a sidearm. The Cary cell was attached to the sidearm through a teflon stopcock. The Cary cell and stopcock assembly are shown in Fig. 23. After the final cycle of degassing, the flask was tipped so that the sample solution flowed through the sidearm into the Cary cell. The stopcock was then closed off and detached from the sidearm.

The photomultiplier was of the S-20 type (RCA 4463). Metal screens were used for calibration purposes. Oscilloscope traces were recorded on Tri-X Panchromatic 35 mm film. For some slower decaying absorbance changes the Sanborn Type 151 recorder was used for readout.
Figure 23. Cary cell and stopcock assembly for flash photolysis experiments.
CHAPTER 3

STEADY ILLUMINATION EXPERIMENTS

Experiments in Non-Viscous Solvents

When degassed ethanol solutions of chlorophyll plus p-benzoquinone are illuminated with red light in an ESR spectrometer cavity, a five-line ESR signal (ratio of peak heights 1:4:6:4:1) is obtained, indicative of the formation of the semiquinone anion free radical (Venkataraman and Fraenkel 1955, Tollin and Green 1962). A typical ESR signal is shown in Fig. 24. This signal occurs immediately upon illumination and goes to zero when the light is turned off. The light-off decay of the signal is too rapid at room temperature to be kinetically analyzed using the ordinary pen recorder. However, by repetitive flash excitation and storage in the memory (400 channels) of a Computer of Average Transients (CAT 400B Mnemotron), one finds that the decay is second order (Fig. 25).

Kinetic analysis of the decay can also be achieved by lowering the temperature sufficiently and employing a somewhat faster recorder (Sanborn Type 151). One again observes the second order nature of the decay (Fig. 26).
Figure 24. Five-line ESR signal of benzosemiquinone anion. Chlorophyll $a$ (4 x 10$^{-5}$M); benzoquinone (1 x 10$^{-3}$M) in ethanol. Illuminated with red light at room temperature.
Light-off decay of ESR signal from CAT memory.

Second order plot of light-off decay of ESR semiquinone signal from CAT memory.

Figure 25. ESR decay curve from CAT memory and second order plot. Chlorophyll a ($4 \times 10^{-5}$M); benzoquinone ($1 \times 10^{-3}$M) in ethanol. Illuminated with red light at room temperature.
Figure 26. Second order plot of light-off decay for ESR signal of semiquinone anion. Chlorophyll a (4 x 10^{-5}M); benzoquinone (1 x 10^{-3}M) in ethanol. Illuminated with red light at -70° to -80°C.
This ESR hyperfine signal is not obtained in the absence of chlorophyll and it is diminished in the presence of air (oxygen). A steady-state concentration of the semiquinone is quickly reached and no further increase in the size of the signal is obtained by prolonged illumination (Tollin and Green 1962). Long term irradiation produces no irreversible chemical changes in this system, as well as in the others to be mentioned below.

Tollin and Green (1962) measured the action spectrum for radical production in the chlorophyll-quinone system in EPA. The results showed that the production of radical was due to light absorbed by the chlorophyll molecule. This semiquinone, then, is the product of a one-electron transfer to the quinone (Fig. 24). One can obtain somewhat larger signals in more basic solvents such as pyridine-water at temperatures around -40°C (Mukherjee, Cho and Tollin 1969).

If a degassed ethanol solution of chlorophyll and p-benzoquinone is made slightly acidic by adding a drop of glacial acetic acid, one obtains a six-line ESR spectrum in the form of three doublets (White and Tollin 1967, see typical spectrum in Fig. 27). This spectrum has been further resolved to show that each of the six lines form a triplet, giving a total 18-line spectrum (Gough 1966). This hyperfine signal represents the protonated semiquinone free radical (Fig. 27).
Figure 27. Six-line ESR signal of the protonated benzo-semiquinone. Chlorophyll a (4 x 10^{-5} M); benzoquinone (1 x 10^{-2} M) in ethanol to which 0.2 ml/10 ml glacial acetic acid was added. Illuminated with red light at room temperature.
In all of the above cases, pheophytin (chlorophyll minus its chelated magnesium ion) may be substituted for chlorophyll with essentially equivalent results. Other porphyrin type molecules (protoporphyrin, hematoporphyrin (Mukherjee et al. 1969), phthalocyanine etc.) will also sensitize the photo-transfer of an electron to a quinone molecule. In these cases one generally obtains smaller signals than with chlorophyll.

The semiquinone radical signal is also obtained if hydroquinone is used in place of benzoquinone (Fujimori and Tavla 1965, Banerjee and Tollin 1966). Here an electron must be withdrawn from the hydroquinone molecule, or in other words a one-electron oxidation of the hydroquinone takes place:

\[
\begin{align*}
&\overset{\text{OH}}{\text{O}} \\
\text{Neutral or Basic Media} &\rightarrow \\
&\overset{\text{O}^\cdot}{\text{O}^-} + e^- + H^+
\end{align*}
\]

If chlorophyll itself is the origin of the electron which reduces the quinone in the first case, and is the recipient of the electron from the hydroquinone in the second, then one might assume that photoexcitation of the chlorophyll molecule is followed by the formation of a chlorophyll free radical in both cases:
However, in all these experiments, no ESR signal attributable to a chlorophyll free radical has been observed (Tollin and Green 1962, Cho and Tollin 1968).

If optical spectra of these systems (neutral and basic media) are examined during illumination, no changes of the chlorophyll bands are found. However, under suitable conditions, a band whose maximum is at 430 nm is produced on illumination (Figs. 28, 29 and 30). This band is identifiable with the benzosemiquinone anion free radical (Linschitz, Bennert and Korn 1954). The best spectra of the semiquinone anion are obtained using hydroquinone rather than benzoquinone. This is due to the instability of benzoquinone in neutral to basic solvents, which is enhanced by illumination. The quinone forms a darkly colored compound and the solution, if the quinone concentration is high enough, eventually becomes opaque (see Fig. 30).
Figure 28. Difference spectrum obtained on illumination of the system: chlorophyll a \((1.5 \times 10^{-5} \text{M})\); hydroquinone \((4 \times 10^{-2} \text{M})\) in pyridine-water \((55:5)\) at \(-30^\circ\text{C}\) to \(-35^\circ\text{C}\).
Figure 29. Difference spectrum obtained on illumination of the system: chlorophyll $a \ (1.5 \times 10^{-5} \text{M})$; hydroquinone $(4 \times 10^{-2} \text{M})$ in ethanol at $-55^\circ \text{C}$ to $-60^\circ \text{C}$.
Figure 30. Difference spectrum obtained on illumination of the system: chlorophyll a (1.5 x 10^{-5} M); benzoquinone (1.0 x 10^{-2} M) in pyridine-water (55:1) at -29°C to -36°C.
Upon turning off the light, one obtains second order decay kinetics for the 430 nm band (Fig. 31). A second order decay constant of $2.4 \times 10^5 \text{ sec}^{-1} \text{M}^{-1}$ is obtained in the case of hydroquinone in pyridine-water. Although this is about twice the value found by Mukherjee et al. (1969) for the second order decay of the ESR signal obtained from this system, the two rate constants are probably the same within experimental error.

An obvious second order decay process for the systems we have been describing is a recombination of chlorophyll and quinone radicals. In the case of benzoquinone:

$$\text{Chl}^+ + \text{Q}^- \longrightarrow \text{Chl} + \text{Q}$$

In the hydroquinone system, one would also need the participation of a proton:

$$\text{ChlH}^+ + \text{Q}^- + \text{H}^+ \longrightarrow \text{Chl} + \text{H}_2\text{Q}$$

However, in view of the inability to observe corresponding changes (either optical or ESR) of chlorophyll in the aforementioned systems, simple recombination may not be the major decay mechanism. Furthermore, convincing evidence that the quinone radicals decay by disproportionation has been reported (Mukherjee et al. 1969, Cho and Tollin 1968). Because of this, Cho and Tollin (1968) favored a radical
Figure 31. Second order plot of light-off decay of 430 nm band of the benzosemiquinone anion. Chlorophyll a (1.5 x 10^{-3} M); hydroquinone (4 x 10^{-2} M) in pyridine-water (55:5) at -30° to -35° C.

Calculation of second order decay constant:

slope = \frac{129 \text{ A}^{-1}}{4 \text{ sec}} = 32.25 \text{ A}^{-1} \text{ sec}^{-1} \text{ M}^{-1}

Using \( \epsilon_{430} = 7400 \text{ A/M} \)

\( k_2 = 32.25 \times 7400 \text{ sec}^{-1} \text{ M}^{-1} \)

\( k_2 = 2.4 \times 10^5 \text{ sec}^{-1} \text{ M}^{-1} \)
disproportionation mechanism whereby disproportionation of chlorophyll radicals occurred at a much faster rate than the disproportionation of semiquinone radicals:

\[
\text{Chl}^+ + \text{Chl}^+ \rightarrow \text{Chl} + \text{Chl}^{2+}
\]

\[
Q^- + Q^- + 2H^+ \rightarrow Q + H_2Q
\]

\[
\text{Chl}^{2+} + H_2Q \rightarrow \text{Chl} + Q + 2H^+
\]

The steady-state concentration of chlorophyll radical during illumination is postulated to be too small to detect. The present failure to observe optical signals due to chlorophyll bleaching, however, would not be consistent with the formation of a species such as Chl^{2+}.

Degassed ethanol solutions of chlorophyll and quinone can be made to show optical changes of the chlorophyll bands on steady illumination (Linschitz and Rennert 1952). This requires lowering the temperature close to the freezing point of ethanol (-117°C). At these low temperatures the ethanol is highly viscous (according to the Handbook of Chemistry and Physics (1968), 44.0 centipoises at -98°C). Because of condensation and cracking of the solvent, it is difficult to get good data from such experiments. Fig. 32 shows a light-dark difference spectrum obtained with a chlorophyll-benzoquinone system in ethanol at low
Figure 32. Difference spectrum obtained on illumination of the system: chlorophyll a ($1.8 \times 10^{-5}$M); benzoquinone ($1.0 \times 10^{-2}$M) in ethanol at -70° to -90°C.
temperatures. Bleaching of the chlorophyll bands is quite
evident. Positive changes in the 400-500 nm region of the
spectrum may be due in part to semiquinone and also to a
modified chlorophyll species. Hydroquinone under these
conditions gives only the semiquinone positive absorbance
change at 430 nm.

While kinetic analysis of these low temperature
data is difficult, one experiment giving data for the 430 nm
bleaching at about -100°C gave decay kinetics which plotted
first order with a constant of 1.8 sec⁻¹ (half-life = 380
msec) (Fig. 33).

From these results it is possible to conclude that
low temperatures and/or high viscosities stabilize a
bleached chlorophyll species. This is in agreement with
the work of Linschitz and Rennert (1952) and of Krasnovskii
and Drozdova (1964).
Figure 33. First order plot of light-off decay of bleaching at 430 nm. Chlorophyll a (1.8 x 10^{-5} M); benzoquinone (1.0 x 10^{-2} M) in ethanol at about -100°C.
Experiments in Viscous Alcoholic Solvents

If higher viscosity is the solvent property which is needed to elicit chlorophyll optical density changes, then by using a more viscous alcohol as solvent, we should be able to avoid going to very low temperatures. Krasnovskii and Drozdova (1964) described experiments in which they investigated chlorophyll optical density changes in the chlorophyll-quinone system using a mixed solvent of glycerol-ethanol. Using their recipe for mixing and degassing the solution, it was possible to obtain changes in optical density in the chlorophyll absorption region with rather slow rise and first order decay kinetics (at temperatures ranging between -55°C to -60°C). A sample of the light-off decay kinetics at 443 nm and -55°C is: first order decay constant, 0.06 ± 0.01 sec⁻¹; half-life, 11.5 sec.

Solutions of chlorophyll alone give slow extensive bleaching of the red band which is to a large extent irreversible, but no absorbance changes can be observed in the blue (Soret) region. Chlorophyll with hydroquinone behaves in a similar fashion as does chlorophyll alone, only the very slow irreversible absorbance changes are observed.
It was decided that glycerol-ethanol and the prescribed method of degassing was not ideal. Large amounts of irreversible bleaching occurred during experiments and, because of the distillation of ethanol from the solvent during degassing, there remained an uncertainty in the ratio of glycerol to ethanol. Therefore, it was difficult to compare results from one experiment to the next.

A possible substitute for glycerol would be cyclohexanol. It is a translucent crystalline solid at room temperature, but with the addition of a small amount of ethanol, it will become a viscous liquid. In order to keep it from crystallizing at low temperatures, some tert-butanol was also added. After much experimentation, it was found that a mixture of cyclohexanol, tert-butanol and ethanol in the ratio 675:200:125 remained a glass at dry ice-alcohol temperatures for at least five hours. However, the stability of this glass at low temperatures was lessened appreciably at high quinone concentrations (\( \geq 10^{-2} \text{M} \)). Determination of the viscosity of the cyclohexanol-tert-butanol-ethanol (CBE) solvent by the dropping sphere method gave a value of 22.6 centipoise at 23°C.

Spectral Changes

Chlorophyll and benzoquinone in CBE, degassed either by nitrogen gas purging or by refluxing, shows reversible
optical density changes at room temperature similar to those observed with ethanol at very low temperatures (Fig. 34). Bleaching of the chlorophyll Soret, red band and first satellite of the red band is plainly discernable. Also seen is increased optical density in the intermediate spectral region with a maximum at about 460-470 nm and a possible second band at 530 nm. Similar difference spectra are obtained with a variety of quinones (Figs. 35, 36 and 37). The size of the spectral changes is not profoundly affected as one goes from -60°C to 0°C, but in the nitrogen gas purged samples at room temperature and higher, signals deteriorate appreciably (although not in the refluxed samples). This is probably due to photo-oxidation of chlorophyll by residual oxygen.

Comparison of samples degassed by purging with those degassed by refluxing shows that the signal sizes are larger and the kinetics slower in the latter system. As an example, in a solution of chlorophyll a (2 x 10^{-5}M) and p-benzoquinone (1.0 x 10^{-2}M) at room temperature; for purged CBE, \( \Delta A(\text{at } 430 \text{ nm}) = -0.0023, k_1 = 1.4 \text{ sec}^{-1} \); for refluxed CBE, \( \Delta A(\text{at } 430 \text{ nm}) = -0.017, k_1 = 0.022 \text{ sec}^{-1} \). Determination of viscosity (by the dropping sphere method) shows no detectable difference between the two samples and so these results are probably due to a lower concentration of oxygen in the refluxed solvent.
Figure 34. Difference spectrum obtained on illumination of the system: chlorophyll a (2 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE at room temperature.
Figure 35. Difference spectrum obtained on illumination of the system: chlorophyll a \((2.0 \times 10^{-5} \text{M})\); 2,5-dichloro-p-benzoquinone \((1.1 \times 10^{-5} \text{M})\) in CBE (N\(_2\) purged), at \(-54^\circ\text{C}\) to \(-63^\circ\text{C}\).
Figure 36. Difference spectrum obtained on illumination of the system: chlorophyll a (2.2 x 10^-5M); methyl-p-benzoquinone (1.0 x 10^-2M) in CBE (N2 purged), at -40° to -45°C.
Figure 37. Difference spectrum obtained on illumination of the system chlorophyll A (2.1 x 10^-5 M) + 2,3,5-trimethyl-p-benzoquinone (1.1 x 10^-2 M) in CBE (N2 purged), at -45 to -50 C.
Kinetic Comparisons and ESR Signals

We have measured the rise and decay kinetics at a variety of wavelengths for several chlorophyll-quinone systems in CBE (Tables 1, 2, 3 and 4). The decay is first order (Figs. 38, 39 and 40) for all wavelength regions and the decay constants are found to be approximately equal (Table 5). The rise kinetics are more complicated and appear to be at least biphasic (Fig. 41). This is especially evident in the benzoquinone system. Both rise and decay kinetics vary with quinone species (see below for further discussion). Quinone concentration (relative to chlorophyll) affects the shape of the rise curve (Figs. 42 and 43) but does not seem to affect the light-off decay rate. There also seems to be no effect on the decay rate of the ESR signal (Table 6).

In contrast with experiments using ethanol and pyridine solvents, ESR signals obtained in CBE show no hyperfine structure (Fig. 44). The decay kinetics are again first order (Figs. 38, 39 and 40), but the decay constant is significantly faster than for the optical changes. Differences are also readily apparent in the rise kinetics. The ESR signal reaches a steady state much more rapidly than does the optical signal and no indication is present of a biphasic process (Figs. 45, 46, 47 and 48).
Table 1. First order light-off and light-on constants for various wavelengths and ESR signal. System: chlorophyll a (2 x 10^{-5}M); 2,5-dichloro-p-benzoquinone (1.1 x 10^{-3}M) in CBE (refluxed) at rm. temp.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Light-off decay (sec^{-1})</th>
<th>Light-on rise (sec^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>430</td>
<td>0.0040</td>
<td>0.15</td>
</tr>
<tr>
<td>460</td>
<td>0.0049</td>
<td>0.12</td>
</tr>
<tr>
<td>630</td>
<td>0.0042</td>
<td>0.055</td>
</tr>
<tr>
<td>660 (670)</td>
<td>0.0049</td>
<td>0.045</td>
</tr>
<tr>
<td>ESR</td>
<td>0.0077</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. First order light-off and light-on constants for various wavelengths and ESR signal. System: chlorophyll a ($2 \times 10^{-5}$M); p-benzoquinone ($1.0 \times 10^{-2}$M) in CBE (refluxed), at rm. temp.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Light-off decay (sec$^{-1}$)</th>
<th>Light-on rise* (sec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>fastest</td>
</tr>
<tr>
<td>430</td>
<td>0.0090</td>
<td>0.97</td>
</tr>
<tr>
<td>460</td>
<td>0.0096</td>
<td>1.03</td>
</tr>
<tr>
<td>630</td>
<td>0.0094</td>
<td></td>
</tr>
<tr>
<td>660(670)</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>ESR</td>
<td>0.080</td>
<td></td>
</tr>
</tbody>
</table>

*This is assumed to be triphasic in these calculations, but the physical reality of a third component is not certain.
Table 3. First order light-off and light-on constants for various wavelengths and ESR signal. System: chlorophyll a (2 x 10^{-2}M); methyl-p-benzoquinone (0.9 x 10^{-2}M) in CBE (refluxed), at rm. temp.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Light-off decay (sec^{-1})</th>
<th>Light-on rise (sec^{-1})</th>
<th>Fastest</th>
<th>Medium</th>
<th>Slowest</th>
</tr>
</thead>
<tbody>
<tr>
<td>430</td>
<td>0.072</td>
<td>--</td>
<td>0.88</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>460</td>
<td>0.077</td>
<td>--</td>
<td>0.76</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>630</td>
<td>0.083</td>
<td>--</td>
<td>0.87</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>ESR</td>
<td>0.16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Table 4.** First order light-off and light-on constants for various wavelengths and ESR signal. System: chlorophyll a (2 x 10^{-5}M); 2,5-dimethyl-benzoquinone (0.6 x 10^{-2}M) in CBE (refluxed), at rm. temp.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Light-off decay (sec^{-1})</th>
<th>Light-on rise (sec^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>430</td>
<td>0.32</td>
<td>fastest 1.25 medium 0.61</td>
</tr>
<tr>
<td>460</td>
<td>0.23</td>
<td>fastest 1.78 medium 0.68</td>
</tr>
<tr>
<td>630</td>
<td>0.20</td>
<td>fastest 1.05 medium 0.38</td>
</tr>
<tr>
<td>ESR</td>
<td>0.23</td>
<td></td>
</tr>
</tbody>
</table>
Figure 38. First order plot of light-off decay for bleaching at 630 nm and ESR signal. System: chlorophyll a (2 x 10^{-5} M); 2,5-dichloro-p-benzoquinone (1.1 x 10^{-3} M) in CBE (refluxed), at rm. temp.
Figure 39. First order plot of light-off decay for bleaching at 670 nm and ESR signal. System: chlorophyll a (2 x 10^{-3}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (refluxed) at rm. temp.
Figure 40. First order plot of light-off decay for bleaching at 430 nm, rise at 460 nm and ESR signal. System: chlorophyll a (2 x 10^{-5}M); methyl-p-benzoquinone (0.9 x 10^{-2}M) in CBE (refluxed) at rm, temp.
Table 5. First order light-off decay constants for various wavelengths and ESR signal. System: chlorophyll a (2 x 10^{-5} M); p-benzoquinone (1.0 x 10^{-2} M) in CBE, at rm. temp.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Absorbance Change (steady-state)</th>
<th>Decay Constant (sec^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>430</td>
<td>-0.017</td>
<td>0.017</td>
</tr>
<tr>
<td>443</td>
<td>-0.0054±0.0003</td>
<td>0.022</td>
</tr>
<tr>
<td>450</td>
<td>+0.0058</td>
<td>0.023</td>
</tr>
<tr>
<td>460</td>
<td>+0.0075±0.0003</td>
<td>0.024</td>
</tr>
<tr>
<td>474</td>
<td>+0.0065±0.0003</td>
<td>0.027</td>
</tr>
<tr>
<td>629</td>
<td>-0.0058±0.0001</td>
<td>0.030</td>
</tr>
<tr>
<td>650</td>
<td>-0.0085±0.0002</td>
<td>0.026</td>
</tr>
<tr>
<td>660</td>
<td>-0.020</td>
<td>0.030</td>
</tr>
<tr>
<td>671</td>
<td>-0.017±0.000</td>
<td>0.038</td>
</tr>
<tr>
<td>683</td>
<td>-0.0040±0.0000</td>
<td>0.045</td>
</tr>
<tr>
<td>ESR</td>
<td>0.066</td>
<td></td>
</tr>
</tbody>
</table>
Figure 41. Plot showing multiphasic character of light-on rise curve:
Rise velocity (Δabsorbance/sec) versus concentration (Δabsorbance) for
bleaching at 430 nm. System: chlorophyll a (2 x 10^{-5} M); p-benzoquinone
(1.0 x 10^{-2} M) in CBE (refluxed), at rm. temp.
Figure 42. Optical light-on and light-off curves for the system: chlorophyll a \((1.1 \times 10^{-5} \text{M})\); p-benzoquinone \((2.1 \times 10^{-4} \text{M})\) in CBE (refluxed) at rm. temp.
Figure 43. Optical light-on curves for the system: chlorophyll a (1.9 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (refluxed), at rm. temp.
Table 6. ESR and optical decay constants for various ratios of p-benzoquinone to chlorophyll a for the system chlorophyll a: p-benzoquinone in CBE (refluxed), at rm. temp.

<table>
<thead>
<tr>
<th>molar ratio</th>
<th>ESR decay constant (sec⁻¹)</th>
<th>optical decay constant (sec⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BQ/Chl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.7</td>
<td>0.14</td>
<td>0.0090 (430 nm)</td>
</tr>
<tr>
<td>18.8</td>
<td>0.076</td>
<td>0.026 (430 nm)</td>
</tr>
<tr>
<td>19.1</td>
<td></td>
<td>0.0071 (620 nm)</td>
</tr>
<tr>
<td>21.3</td>
<td></td>
<td>0.0078 (620 nm)</td>
</tr>
<tr>
<td>62.5</td>
<td>0.070</td>
<td></td>
</tr>
<tr>
<td>111</td>
<td>0.057</td>
<td>0.017 (650 nm)</td>
</tr>
<tr>
<td>427</td>
<td>0.078</td>
<td>0.017 (430 nm)</td>
</tr>
<tr>
<td>526</td>
<td>0.066</td>
<td>0.017 (430 nm)</td>
</tr>
<tr>
<td>910</td>
<td></td>
<td>0.0091 (430 nm)</td>
</tr>
</tbody>
</table>
Figure 44. ESR signals obtained from steady illumination of chlorophyll-quinone systems.
Figure 45. ESR and optical light-on and light-off curves. System: chlorophyll a (1.9 x 10^{-5} M); 2,5-dichloro-p-benzoquinone (1.1 x 10^{-3} M) in CBE (refluxed), at rm. temp.
Figure 46. ESR and optical light-on and light-off curves. System: chlorophyll a (1.9 x 10^{-5} M); p-benzoquinone (1.0 x 10^{-2} M) in CBE (refluxed) at rm. temp.
Figure 47. ESR and optical light-on and light-off curves. System: chlorophyll a (1.9 x 10^{-5}M); methyl-p-benzoquinone (0.9 x 10^{-2}M) in CBE (refluxed), at rm. temp.
Figure 48. ESR and optical light-on and light-off curves. System: chlorophyll a (1.9 x 10^{-5} M); 2,5-dimethyl-p-benzoquinone (0.6 x 10^{-2} M) in CBE (refluxed), at rm. temp.
A further difference exists between the optical and ESR results. The ESR decay is markedly affected by quinone structure whereas the rise is much less so. In the optical experiments, both rise and decay depend on the quinone used.

These results demonstrate that at least two species are being generated by light in the chlorophyll-quinone mixtures. One of these is produced rapidly and is paramagnetic whereas the more slowly generated species is not. Both decay by first order kinetics.* It is not possible to decide from kinetics whether these products are being formed by a sequential or a parallel set of reactions.

Illumination Intensity

Intensity of illumination affects both the size of the signals (optical changes and ESR) and the initial velocity of the light-on signal. A plot of either initial velocity (Fig. 49) or signal height (Fig. 50) versus relative intensity gives a straight line for the reactions in CBE. This linear relationship between initial velocity and light intensity shows that the reaction rate is determined by the concentration of excited state species.

*However, the fact that only a single decay process is observed in the optical experiments may simply reflect the relative concentrations of the two types of species.
Figure 49. Plot of initial velocity of bleaching at 430 nm versus relative intensity of illumination. System: chlorophyll a (1.8 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-3}M) in CBE (refluxed), at rm. temp.
Figure 50. Plot of signal height versus relative intensity for change in absorbance at 430 nm, for the system: chlorophyll a (1.8 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-3}M) in CBE (refluxed), at rm. temp.
The pH of the solvent has a profound effect on the size of the chlorophyll difference spectrum. Adding 0.2 ml of glacial acetic acid to 10 ml of CBE causes a large increase in the magnitude of the absorbance changes over those observed in untreated CBE (Fig. 51). A linear relationship exists between the log of the amount of acetic acid added and the size of the optical signal (Fig. 52). These amounts of acid are not sufficient to cause appreciable pheophytinization. On the other hand, adding 0.5% pyridine causes decreases in bleaching at 430 nm and 660 nm as well as a decrease in the positive signal at 474 nm. Acetic acid has a similar effect in degassed ethanol at low temperatures, although 1% pyridine does not (Fig. 53).

Light-off decay kinetics of the various signals are also affected by pH. In general, a large decrease in velocity is observed as the solutions go from base to neutral to acid (Table 7). A much smaller increase in the initial rate of light-on rise at 430 nm is found in CBE as the amount of acetic acid added increases (Fig. 54). However, the initial rates in degassed ethanol at low temperature seem to be the same in the presence of acetic acid or pyridine (Fig. 55).

If the solvent is made strongly basic (e.g., by saturating it with potassium acetate or by adding larger
Figure 51. Absorbance changes at three wavelengths for the system: chlorophyll a (1.5 x 10^{-5} M); p-benzoquinone (1.0 x 10^{-2} M) in CBE (N₂ purged) at -64°C, to which has been added: A = plus acetic acid (0.2 ml/10 ml)
B = plus 0.5 % pyridine
N = no additive
Figure 52. Plot of the change in $\Delta$ absorbance (at 430 nm) with increase of acidity (log plot). System: chlorophyll a ($1.7 \times 10^{-5}$M); p-benzoquinone ($1.0 \times 10^{-2}$M) in CBE (refluxed) at rm. temp.
Figure 53. Absorbance changes at 430 nm (4 sec after light on). System: chlorophyll a (1.6 x 10^{-5} M), p-benzoquinone (1.0 x 10^{-2} M) in degassed ethanol at -97°C.
Table 7. First order light-off decay constants as affected by added acid and base. System: chlorophyll a \((1.5 \times 10^{-5} \text{M})\) p-benzoquinone \((1.0 \times 10^{-2} \text{M})\) in CBE (N\(_2\) purged) at \(-64^\circ\text{C}\).

<table>
<thead>
<tr>
<th>additive</th>
<th>(430\text{ nm bleaching (sec}^{-1}))</th>
<th>(474\text{ nm rise (sec}^{-1}))</th>
<th>(663\text{ nm bleaching (sec}^{-1}))</th>
<th>ESR signal (sec}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 ml/10ml acetic acid</td>
<td>0.024</td>
<td>0.020</td>
<td>0.030</td>
<td>0.012</td>
</tr>
<tr>
<td>no additive</td>
<td>0.22</td>
<td>0.18</td>
<td>--</td>
<td>0.38</td>
</tr>
<tr>
<td>0.5% pyridine</td>
<td>0.36</td>
<td>0.31</td>
<td>0.25</td>
<td>--</td>
</tr>
</tbody>
</table>
Figure 54. Change in initial velocity with increased added acetic acid. System: chlorophyll a (1.7 x 10^{-5} M); p-benzoquinone (1.0 x 10^{-2} M) in CBE (refluxed), at rm. temp.
Figure 55. Initial light-on rise velocities at 430 nm for the system: chlorophyll a (1.6 x 10^{-5} M); p-benzoquinone (1.0 x 10^{-2} M) in degassed ethanol at -97°C.
amounts of pyridine), one will obtain no chlorophyll opti­
cal changes and only the five-line ESR semiquinone signal, which decays quickly.

Temperature Dependence

The activation energies for signal decay, obtained from Arrhenius plots (Figs. 56 to 64), for chlorophyll a plus p-benzoquinone in viscous alcoholic solvents range between 10-14 kcal/mole. pH does not appear to have much effect on this value. Ethanol plus a trace of acetic acid gives a lower value of 8-9 kcal/mole. Thus, less energy is required for decay in a non-viscous solvent. Lower poten­
tial quinones give lower activation energies (for trimethyl­
benzoquinone it is 2.5 kcal/mole). These results are summarized in Table 8.

Quenching Experiments

All of the solutions used for these experiments are degassed to eliminate dissolved oxygen, inasmuch as oxygen is an efficient quencher of the triplet excited states of organic molecules. Oxygen quenching can be easily demon­
strated in the present systems by taking a degassed chlorophyll-quinone solution, which gives ESR signals or optical density changes on illumination, and saturating it with oxygen (by bubbling O₂ through the solution for about
Figure 56. Arrhenius plot: log k (first order decay constant) versus 1/T for positive absorbance changes measured at 474 nm. System: chlorophyll a (1.7 x 10^-5 M); p-benzoquinone (1.0 x 10^-2 M) in CBE (N₂ purged).
Figure 57. Arrhenius plot: log k (first order decay constant) versus 1/T for bleaching at 430 nm. System: chlorophyll a (1.8 x 10^{-5}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (N₂ purged).
Figure 58. Arrhenius plot: log k (first order decay constant) versus 1/T for bleaching at 663 nm. System: chlorophyll a (1.7 x 10^{-5} M); p-benzoquinone (6.3 x 10^{-3} M) in glycerol-ethanol (degassed).
Figure 59. Arrhenius plot: log k (first order decay constant) versus 1/T for bleaching at 430 nm. System: chlorophyll a (1.8 x 10^{-3}M); p-benzoquinone (1.0 x 10^{-2}M) in degassed ethanol plus acetic acid (0.3 ml/100 ml).
Figure 60. Arrhenius plot: log k (first order decay constant) versus 1/T for ESR signal decay. System: chlorophyll a (1.8 x 10^{-5} M); p-benzoquinone (1.0 x 10^{-2} M) in CBE (N₂ purged).
Figure 61. Arrhenius plot: log k (first order decay constant) versus 1/T for ESR signal decay. System: chlorophyll a (1.8 x 10^{-5} M); p-benzoquinone (1.0 x 10^{-2} M) in CBE (N2 purged) plus acetic acid (0.2 ml/10 ml).

\[ E_a = 12.8 \text{ kcal/mole} \]
Figure 62. Arrhenius plot: log $k$ (second order decay constant) versus $1/T$ for ESR signal decay. System: chlorophyll $a$ ($3.0 \times 10^{-4}$M); p-benzoquinone ($1.0 \times 10^{-2}$M) in CBE ($N_2$ purged) plus 1% potassium acetate saturated CBE.
Figure 63. Arrhenius plot: log k (first order decay constant) versus 1/T for ESR signal decay. System: chlorophyll a (1.8 \times 10^{-3}M); p-benzoquinone (1.0 \times 10^{-2}M) in degassed ethanol plus acetic acid (0.3 ml/100 ml).

\[ E_a = 8.8 \text{ kcal/mole} \]
Figure 64. Arrhenius plot: log k (first order decay constant) versus 1/T for ESR signal decay. System: chlorophyll a ($4.4 \times 10^{-5}$M); trimethyl-p-benzoquinone ($1.0 \times 10^{-2}$M) in CBE (refluxed).
Table 8. Activation energies for chlorophyll-quinone systems (from Arrhenius plots)

<table>
<thead>
<tr>
<th></th>
<th>chl conc. (molar x 10^{-5})</th>
<th>quinone conc. (molar x 10^{-2})</th>
<th>solvent</th>
<th>activation energy (kcal/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>optical</td>
<td>1.7</td>
<td>BQ</td>
<td>1.0</td>
<td>CBE(p)*</td>
</tr>
<tr>
<td></td>
<td>1.8</td>
<td>BQ</td>
<td>1.0</td>
<td>CBE(p)</td>
</tr>
<tr>
<td></td>
<td>1.7</td>
<td>BQ</td>
<td>0.63</td>
<td>glycerol-ethanol</td>
</tr>
<tr>
<td></td>
<td>1.8</td>
<td>BQ</td>
<td>1.0</td>
<td>ethanol HOAc</td>
</tr>
<tr>
<td>ESR</td>
<td>1.8</td>
<td>BQ</td>
<td>1.0</td>
<td>CBE(p)</td>
</tr>
<tr>
<td></td>
<td>1.8</td>
<td>BQ</td>
<td>1.0</td>
<td>CBE(p) HOAc</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>BQ</td>
<td>1.0</td>
<td>CBE(p) KOAc</td>
</tr>
<tr>
<td></td>
<td>1.8</td>
<td>BQ</td>
<td>1.0</td>
<td>ethanol HOAc</td>
</tr>
<tr>
<td></td>
<td>4.4</td>
<td>TMBQ</td>
<td>1.0</td>
<td>CBE(r)</td>
</tr>
</tbody>
</table>

*(p) = purged; (r) = refluxed; HOAc = acetic acid added; KOAc = potassium acetate added.
one minute). After such treatment, one observes no ESR signal and no optical density changes. By injecting varying small amounts of oxygen into a number of samples, a correspondence can be demonstrated between the decrease in absorbance changes at 430 nm and the amount of oxygen injected. This decrease is also observed in the initial velocity of the light-on signal (Figs. 65 and 66). These results suggest that the chlorophyll triplet is the photoreactive species in producing both the ESR signal and the absorbance changes.

Like oxygen, β-carotene, a compound which specifically quenches the triplet excited state of chlorophyll (Fujimori and Livingston 1957), shows quenching in these experiments. Steady state ESR signal size and magnitude of absorbance changes are dependent on the concentration of β-carotene (Figs. 67 to 70). Stern-Volmer plots can be made from these data. However, the nature of such a plot will depend on the decay kinetics. Inasmuch as the decay processes in the present work are first order, the proper form of the Stern-Volmer equation is:

\[
\frac{P}{P_0} = 1 + \frac{k_2 [Q]}{k_1 + k_3 [Q]}
\]
Figure 65. Plot of Δabsorbance at 430 nm versus oxygen injected (amount of bleaching after 3 sec). System: chlorophyll a (1.3 x 10^{-5}M); p-benzoquinone (3 x 10^{-4}M) at rm. temp., in CBE (refluxed).
Figure 66. Plot of initial velocity versus oxygen injected for bleaching at 430 nm. System: chlorophyll $a$ ($1.3 \times 10^{-5} \text{M}$); p-benzoquinone ($3 \times 10^{-4} \text{M}$) in CBE (refluxed) at rm. temp.
Figure 67. Plot of steady-state $\Delta$absorbance at 630 nm versus concentration of $\beta$-carotene and Stern-Volmer plot of the same system: chlorophyll a ($1.8 \times 10^{-5}$M); methyl-p-benzoquinone ($8.2 \times 10^{-3}$M) in CBE (refluxed) at room temp.
Figure 68. Plot of steady-state ESR signal height versus concentration of \( \beta \)-carotene and Stern-Volmer plot of the same system: chlorophyll \( \alpha \) (1.8 x 10\(^{-5}\)M); methyl-p-benzoquinone (8.2 x 10\(^{-5}\)M) in CBE (refluxed), at rm. temp.
Figure 69. Plot of steady-state ESR signal height versus concentration of $\beta$-carotene and Stern-Volmer plot of the same system: chlorophyll a ($1.6 \times 10^{-5}$M); p-benzoquinone ($1.0 \times 10^{-2}$M) in CBE (refluxed), at rm. temp.
Figure 70. Plot of steady-state ESR signal height versus concentration of \( \beta \)-carotene and Stern-Volmer plot of the same system: chlorophyll \( \alpha \) (2.5 \( \times \) \( 10^{-5} \)M); p-benzoquinone (3.0 \( \times \) \( 10^{-5} \)M) in CBE (refluxed) at rm. temp.
where $P = \text{concentration of photoproduct in absence of quencher}$; $P_q = \text{concentration of photoproduct in presence of quencher}$; $[q] = \text{concentration of quencher}$; $[Q] = \text{concentration of quinone}$; $k_2 = \text{quenching rate constant}$; $k_1 = \text{first order rate constant for triplet decay to ground state}$; $k_3 = \text{second order rate constant for formation of photoproduct by reaction with quinone}$.

Thus, a plot of the reciprocal of photoproduct concentration versus quencher concentration should be a straight line. Although the data are not of the highest quality, the fact that Stern-Volmer kinetics appear to be obeyed indicates that the $\beta$-carotene is acting at the excited state level, and thus that the chlorophyll triplet state is the photoreactive species.

From flash photolysis experiments on $\beta$-carotene quenching of the photobleaching at 430 nm (Fig. 71), one can obtain minimum values for $k_1$ and $k_2$ (see below for further discussion of flash experiments). From a plot of the log of the absorbance change versus time for chlorophyll a alone in CBE (Fig. 72):

$$k_1 \geq 2.3 \times 10^3 \text{ sec}^{-1}$$

From the plot for chlorophyll a plus $\beta$-carotene in CBE (Fig. 72),
Figure 71. Decay of bleaching at 430 nm (oscilloscope traces from flash photolysis experiments) for the system: chlorophyll a (2.5 x 10^{-5}M) in CBE (refluxed), at rm. temp.
Figure 72. First order decay plots of flash excited bleaching at 430 nm for the system: chlorophyll a \((2.5 \times 10^{-5} \text{M})\) in CBE (refluxed) at rm. temp., with and without \(\beta\)-carotene \((1.0 \times 10^{-5} \text{M})\).
\[ k_1 + k_2[q] \geq 10.2 \times 10^3 \text{ sec}^{-1} \]

therefore, since:

\[ k_1 + k_2[q] - k_1 = k_2[q] \]

\[ k_2[q] \geq (10.2 - 2.3) \times 10^3 \]

\[ \geq 7.9 \times 10^3 \text{ sec}^{-1} \]

\[ k_2 \geq \frac{7.9 \times 10^3 \text{ sec}^{-1}}{[q]} \]

where: \([q] = 1.0 \times 10^{-5} \text{M}; \text{ therefore:} \]

\[ k_2 \geq 7.9 \times 10^8 \text{ (M sec)}^{-1} \]

\(k_1\) and \(k_2\) from this sample calculation can be compared to values for \(k_1\) obtained by Linschitz and Sarkanan (1958) of 670 sec\(^{-1}\) in pyridine and 440 sec\(^{-1}\) in benzene and a value for \(k_2\) of \(1.3 \times 10^9 \text{ (M sec)}^{-1}\) obtained by Fujimori and Livingston (1957) for \(\beta\)-carotene in benzene.

For three determinations at 430 nm, our values are:

\[ k_1 \geq 2.3 \pm 0.0 \times 10^3 \text{ sec}^{-1} \text{ and } k_2 \geq 7.5 \pm 0.5 \times 10^8 \text{ (M sec)}^{-1} \].

These numbers allow a minimum value for \(k_3\) to be obtained from the slope of the Stern-Volmer plots.

Using \(k_1\) and \(k_2\) obtained here, minimum values for \(k_3\)'s were calculated from Stern-Volmer plots for four experiments in CBE. The results are shown in Table 9. A sample
Table 9. Calculated $k_3$'s from optical and ESR experiments at room temp. in CBE (refluxed).

<table>
<thead>
<tr>
<th>Chl conc. (molarity x 10^{-5})</th>
<th>quinone</th>
<th>parameter</th>
<th>calculated $k_3$ (M sec)^{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>benzo-quinone (3 x 10^{-4} M)</td>
<td>initial velocity, absorbance at 460 nm</td>
<td>$7.3 \pm 0.8 \times 10^8$</td>
</tr>
<tr>
<td>2.5</td>
<td>benzo-quinone (3 x 10^{-4} M)</td>
<td>ESR signal height</td>
<td>$6.5 \pm 2.2 \times 10^8$</td>
</tr>
<tr>
<td>1.8</td>
<td>methyl benzo-quinone (8.2 x 10^{-3} M)</td>
<td>absorbance change at 630 nm</td>
<td>$0.55 \pm 0.66 \times 10^7$</td>
</tr>
<tr>
<td>1.8</td>
<td>methyl benzo-quinone (8.2 x 10^{-3} M)</td>
<td>ESR signal height</td>
<td>$1.5 \pm 0.6 \times 10^7$</td>
</tr>
</tbody>
</table>
calculation for the first value in Table 9 is as follows:

The Stern-Volmer plot of Fig. 73 gives the reciprocal of initial velocity versus \( \beta \)-carotene concentration. The slope of this line is:

\[
\Delta \frac{1}{v_1} \quad \Delta [\alpha] = \frac{2.33 \pm 0.55 \times 10^2}{10 \times 10^{-5}} \quad \text{(absorbance/sec)}^{-1} \quad \text{M}
\]

Intercept: \( \frac{1}{v_{10}} = 6.95 \pm 0.55 \times 10^2 \quad \text{(absorbance/sec)}^{-1} \)

Therefore:

\[
\Delta \frac{v_{10}}{v_1} \quad \Delta [\alpha] = \frac{k_2}{k_1 + k_3[\alpha]}
\]

\[
= \frac{2.33}{6.95} \quad (\pm 7.8 \% \text{ error}) \quad \frac{10 \times 10^{-5}}{\text{M}} = 3.4 \pm 0.3 \times 10^3 \quad \text{M}^{-1}
\]

Using \( k_1 \) and \( k_2 \) obtained from our flash experiments:

\[
3.4 \pm 0.3 \times 10^3 \quad \text{M}^{-1} = \frac{7.5 \pm 0.5 \times 10^8 \quad \text{(M sec)}^{-1}}{2.3 \times 10^3 \quad \text{sec}^{-1} + k_3[\alpha]}
\]

\[
[\alpha] = 3.0 \times 10^{-4} \quad \text{M}, \quad \text{therefore:}
\]

\[
2.3 \times 10^3 \quad \text{sec}^{-1} + k_3(3.0 \times 10^{-4} \quad \text{M}) = \frac{7.5 \pm 0.5 \times 10^8 \quad \text{sec}^{-1}}{3.4 \pm 0.3 \times 10^3}
\]
Figure 73. Plot of initial velocity at 460 nm versus concentration of β-carotene and Stern-Volmer plot of the same system: chlorophyll a (2.5 x 10^{-5}M); p-benzoquinone (3.0 x 10^{-5}M) in CBE (refluxed) at rm. temp.
$$k_3 = \frac{2.20 \pm 0.24 \times 10^5 - 0.023 \times 10^5}{3.0 \times 10^{-4}} \text{ (M sec)}^{-1}$$

$$k_3 = \frac{2.18 \pm 0.24 \times 10^5}{3.0 \times 10^{-4}} \text{ (M sec)}^{-1}$$

$$k_3 = 7.3 \pm 0.8 \times 10^8 \text{ (M sec)}^{-1}$$

Much smaller constants have been obtained by Mukherjee et al. (1969) from ESR measurements of quinone radical formation ($k_3 = 4.85 \pm 0.07 \times 10^4$ (M sec)$^{-1}$ for chlorophyll a and benzoquinone in ethanol at $-40^\circ$C). This suggests that the present experiments are measuring the formation of a precursor of the semiquinone.

Under steady-state conditions, in the presence of quinone, $\beta$-carotene also undergoes a slow chlorophyll-sensitized photobleaching. If one looks at a wavelength where $\beta$-carotene absorbs (e.g. 460 nm), one observes a biphasic change (Fig. 74). On initiation of illumination, the positive change represents chlorophyll photoproduction formation. After about four seconds, the absorbance decreases steadily due to $\beta$-carotene bleaching. This bleaching is reversible in the dark, but at a much slower rate (the rate is approximately steady at $5 \times 10^{-3}$ $\Delta$absorbance/minute (460 nm), or about $5 \times 10^{-7}$ moles $\beta$-carotene per liter/minute). In the absence of quinone, $\beta$-carotene bleaching is very much reduced. This suggests that the
Figure 74. Light-on and light-off absorbance changes as a function of time at 460 nm for the system: chlorophyll a (2.0 x 10^-5 M); methyl-p-benzoquinone (8.2 x 10^-3 M); β-carotene (1 x 10^-4 M) in CBE (refluxed) at rm. temp.
bleaching involves a reaction between $\beta$-carotene and either the quinone radical or the chlorophyll photoproduct.

Effect of Porphyrin and Quinone Species on Photoreactions

The potential of the quinone affects the magnitude of chlorophyll absorbance changes on steady illumination. In general, the higher the redox potential of the quinone, the larger is the change. The same is true for the ESR signals one obtains for these solutions (Fig. 75). Although it is really the one-electron potential which is the parameter linearly related to the electron affinity of the molecule, these redox potentials reflect one-electron potentials and electron affinities insofar as the $pK$ values of the quinols are similar (Peover 1962). Except for the halogenquinone, this is true of the quinones used in our experiments.

The magnesium-chelated porphyrin system of chlorophyll is not at all specific for these photoreactions. Within limits, the chelated metal, the degree of reduction of the porphyrin ring, the nature of the porphyrin side chains and even some of the atoms of the porphyrin ring can be changed without eliminating absorbance changes and ESR signals. Figs. 76-80 give light-dark difference spectra for zinc and cadmium-pheophytin, bacteriochlorophyll, zinc-hematoporphyrin, hematoporphyrin and zinc-phthalocyanine.
Figure 75. Plots of steady-state signal size versus quinone redox potential for bleaching of absorbance at 670 nm and ESR signal height. System: chlorophyll a (1.9 x 10^{-5}M); quinones (5-10 x 10^{-3}M) in CBE (refluxed) at rm. temp.
Figure 76. Difference spectrum obtained on illumination of the system: zinc-pheophytin a (5 x 10^{-5} M); p-benzoquinone (1.0 x 10^{-2} M) in CBE (refluxed) at rm. temp.
Figure 77. Difference spectrum obtained on illumination of the system: cadmium-pheophytin a (ca. 1 x 10^{-5}M)(equilibrium mixture with pheophytin a); p-benzoquinone (1.0 x 10^{-2}M) in CBE (refluxed) at rm. temp.
Figure 78. Difference spectrum obtained on illumination of the system: bacteriochlorophyll (4 x 10^{-6}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (refluxed) at rm. temp.
Figure 79. Difference spectra obtained on illumination of the systems:

- ◯— zinc-hematoporphyrin (ca. $10^{-5}$ M); p-benzoquinone ($1.0 \times 10^{-2}$ M) in CBE (refluxed) at rm. temp.

- △— hematoporphyrin ($1.5 \times 10^{-4}$ M); hydroquinone ($4 \times 10^{-2}$ M) in pyridine-water (55:5) at $-45^\circ$C.

- ▽— hematoporphyrin ($1.5 \times 10^{-4}$ M); p-benzoquinone ($1.0 \times 10^{-2}$ M) in CBE (refluxed) at rm. temp.
Figure 80. Difference spectrum obtained on illumination of the system: zinc-phthalocyanine (ca. $10^{-5}$M); p-benzoquinone ($1.0 \times 10^{-2}$M) in CBE (refluxed) at rm. temp.
Structural formulas for these porphyrin systems are given in Figs. 1, 6, 11 and 14; absorption spectra are given in Figs. 7, 8, 12, 13, 15, 17 and 18.

The chelation of the magnesium metal ion in the chlorophyll molecule is necessary for seeing absorbance changes due to chlorophyll. Pheophytin (chlorophyll without the chelated metal) in CBE with benzoquinone gives only very small positive $\Delta$ absorbance in the near u.v. and an ESR signal indicative of the monoprotonated benzoquinone (Fig. 8). As with chlorophyll, it is found that chelation of a metal is necessary for other metal-porphyrin systems, and further, the metal must be similar to magnesium in its electronic orbital structure (i.e., Zn and Cd behave like Mg, whereas the transition metals: Fe, Co, Cu and Ni, although when chelated with pheophytin give absorption spectra similar to chlorophyll, do not elicit absorbance changes or ESR signals. This may be due to paramagnetic quenching of the porphyrin triplet.).

So long as the conjugated system of the porphyrin ring remains intact, porphyrin, dihydrophorpyrin and tetrahydroporphyrin are all effective in these photoreactions. However reduction of the ring (e.g., with sodium borohydride) destroys this ability. Since we have found chelated hematoporphyrin and deuteroporphyrin to be effective, ring 5 and
Figure 81. ESR spectrum of monoprotonated benzosemiquinone obtained on illumination of the system: pheophytin a ($1 \times 10^{-4}$M); p-benzoquinone ($1.0 \times 10^{-2}$M) in CBE (refluxed) at rm. temp.
other porphyrin side chains must not play a major role in these reactions.

Bacteriochlorophyll gives a fast rise and slow second order decay (Fig. 82) in contrast to chlorophyll and other porphyrins investigated. Since benzoquinone is present in an amount of more than a thousand times that of bacteriochlorophyll, the predominant decay reaction must involve two photoproducts reacting together. This interesting difference in the photochemical behavior of chlorophyll and bacteriochlorophyll should be investigated further.

As with chlorophyll $a$, the size of the absorbance change and ESR signal depends on the potential of the quinone. We have plotted the log of the absorbance change and the ESR signal size versus the redox potential of the quinone (Clark 1960) for chlorophyll $a$, zinc-pheophytin, cadmium-pheophytin and zinc-hematoporphyrin. These plots are straight lines (Figs. 83, 84 and 85). In addition, we have plotted these values versus half-wave potentials of the quinones as determined in acetonitrile (Peover 1962) (Figs. 86, 87 and 88). These plots represent the Nernst equation of the photopotential of the porphyrin system for the light intensity employed (approximately $10^5$ ergs per cm$^2$·sec). This photopotential can be estimated from these

*Values in alcohol or water parallel values in acetonitrile except for the halogenquinones (Peover 1962).
Figure 82. Second order plot for the light-off decay of optical and ESR signals. System: bacteriochlorophyll (4 x 10^{-6}M); p-benzoquinone (1.0 x 10^{-2}M) in CBE (refluxed) at rm. temp.
Figure 83. Plot of the log of the absorbance change at 670 nm and ESR signal size versus quinone redox potential for the system: chlorophyll a (1.9 x 10^-5 M); quinones (5-10 x 10^-3 M) in CBE (refluxed) at rm. temp.
Figure 84. Plots of the log of the absorbance change and ESR signal size versus quinone redox potential for systems: zinc-pheophytin \( \text{a} \) (5 x 10\(^{-5}\)M) or cadmium-pheophytin (ca. 1 x 10\(^{-5}\)M); quinones (5-10 x 10\(^{-3}\)M) in CBE (refluxed) at rm. temp.
Figure 85. Plot of the log of the absorbance change and ESR signal size versus quinone redox potential for the system: zinc-hematoporphyrin (ca. $10^{-5}$M), quinones ($5-10 \times 10^{-3}$M) in CBE (refluxed) at rm. temp.
Figure 86. Plot of log of the absorbance change at 670 nm and ESR signal size versus quinone half-wave potential (measured in acetonitrile). System: chlorophyll a (1.9 x 10⁻⁵ M); quinones (5-10 x 10⁻³ M) in CBE (refluxed) at room temp.
Figure 87. Plots of the log of the absorbance change and ESR signal size versus quinone half-wave potential (measured in acetonitrile). Systems: zinc-pheophytin a (5 x 10^{-5}M) or cadmium-pheophytin (ca. 1 x 10^{-5}M); quinones (5-10 x 10^{-3}M) in CBE (refluxed) at rm. temp.
Figure 88. Plot of the log of the absorbance change and ESR signal size versus quinone half-wave potential (measured in acetonitrile). System: zinc-hematoporphyrin (ca. $10^{-5}$M); quinones ($5$-$10$ x $10^{-3}$M) in CBE (refluxed) at rm. temp.
plots. Thus, for chlorophyll a, the \( \Delta \)absorbance representing one-half of the original chlorophyll absorbance at 670 nm will give the photopotential of chlorophyll, since when one-half of the chlorophyll is bleached, the log term in the Nernst equation is zero:

\[
E = E_{ochl} + \frac{RT}{nF} \ln \left( \frac{[Chl]^0}{[Chl]} \right)
\]

The absorbance of the 670 nm red band of chlorophyll for this experiment is 1.57. Half of this is 0.785 and corresponds to a potential of about +0.05 v (SCE) or +0.29 v (NHE) (not shown on the plot of Fig. 86). It is interesting that this value is close to the value of +0.41 v (NHE) given as the maximum half-wave potential for reduction of triplet excited chlorophyll a in the reaction:

\[
Chl^t + Red \rightarrow Chl^- + Red^+ 
\]

(Seely 1966). Photopotentials for the metal porphyrins were similarly determined from Figs. 86, 87 and 88 and are:

<table>
<thead>
<tr>
<th>Metal-porphyrin</th>
<th>Photopotential</th>
</tr>
</thead>
<tbody>
<tr>
<td>chlorophyll a</td>
<td>+0.29 v (NHE)</td>
</tr>
<tr>
<td>zinc-pheophytin a</td>
<td>-0.08</td>
</tr>
<tr>
<td>cadmium-pheophytin a</td>
<td>-0.09</td>
</tr>
<tr>
<td>zinc-hematoporphyrin</td>
<td>+0.40</td>
</tr>
</tbody>
</table>
The redox potential of the quinone is also found to affect the first order light-off signal decay in a manner similar to its effect on signal size. The higher the redox potential of the quinone (i.e. the more positive), the slower is the light-off decay (the smaller the decay constant). Plots of the log of the first order light-off decay constant for optical signals and ESR signals* versus quinone potential also give what appears to be a straight line relationship (Figs. 89, 90 and 91).

The facts that the kinetic properties of the absorbance changes and the ESR signals in the porphyrin-quinone systems depend on both the porphyrin species and the quinone species, and that first order decays are obtained (except for bacteriochlorophyll), strongly suggests that one of the intermediates being observed in these experiments is a complex containing both porphyrin and quinone species. The paramagnetism of this material indicates that one or both of the species is a radical.

One can rationalize these observations within the framework of the idea of photo-induced electron transfer. Thus, two effects will produce a larger steady-state concentration of photoproduct:

*ESR machine response time used (1 sec) limited the meaningful kinetic data only to the slowest decays, i.e. only to the three highest potential quinones with chlorophyll.
Figure 89. Plots of the log of the first order light-off decay constant versus quinone redox potential. System: chlorophyll a (1.9 x 10^{-5}M); quinones (5-10 x 10^{-3}M) in CBE (refluxed) at rm. temp.
Figure 90. Plots of the log of the first order light-off decay constant versus quinone redox potential. Systems: zinc-pheophytin a (5 x 10^{-5}M) or cadmium-pheophytin (1 x 10^{-5}M); quinones (5-10 x 10^{-3}M) in CBE (refluxed) at rm. temp.
Figure 91. Plots of the log of the first order light-off decay constant versus quinone redox potential. System: zinc-hematoporphyrin (ca. $10^{-5}$M); quinones ($5$-$10 \times 10^{-3}$M) in CBE (refluxed) at rm. temp.
1) high rate of radical formation
2) slowness of the back reaction

That we do observe slower decay rates along with larger steady-state radical concentrations tends to implicate the second effect (see straight line plots of \( \log(\Delta \text{absorbance}) \) versus \( \log(\text{decay constant}) \), Figs. 92 and 93). Thus higher redox potential quinones give larger steady-state changes because of a slower back reaction.

If higher rates of radical formation caused the larger steady-state concentrations of photoproduct, this fact would come out in a comparison of initial velocities (which mirror the rate of radical formation). That this is not the case is seen in Table 10 which lists initial velocities for four quinones at three wavelengths. No such trend is found in these figures. If anything, a reverse correlation exists.

---

*The values for initial velocities are obtained from plots of \( \Delta \text{absorbance/sec} \) versus \( \Delta \text{absorbance} \) and extrapolated to zero \( \Delta \text{absorbance} \).
Figure 92. Plot of the log of absorbance change (at 670 nm) versus the log of the first order light-off decay constant. System: chlorophyll a ($1.9 \times 10^{-5}$ M), quinones ($5-10 \times 10^{-3}$ M) in CBE (refluxed) at rm. temp.
Figure 93. Plot of the log of absorbance change (at 409 nm) versus the log of the first order light-off decay constant. System: zinc-hematoporphyrin (ca. $10^{-5}$ M); quinones ($5-10 \times 10^{-3}$ M) in CBE (refluxed) at rm. temp.
Table 10. Initial velocities of light-on rise signal for four quinones at three wavelengths. System: chlorophyll a (1.9 x 10^{-5}M); quinones (5-10 x 10^{-3}M) in CBE (refluxed) at rm. temp.

<table>
<thead>
<tr>
<th>quinone</th>
<th>quinone redox potential</th>
<th>(Δ absorbance/sec) x 10^{-3}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>430 nm</td>
</tr>
<tr>
<td>dichloro-benzoquinone</td>
<td>0.74 volt</td>
<td>4.6</td>
</tr>
<tr>
<td>benzoquinone</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>methyl-benzoquinone</td>
<td>0.65</td>
<td>10</td>
</tr>
<tr>
<td>dimethyl-benzoquinone</td>
<td>0.60</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 4

SUMMARY OF MAJOR CONCLUSIONS
FROM STEADY-STATE EXPERIMENTS

Before proceeding to a discussion of the results of flash photolysis experiments, it is desirable to summarize the major conclusions which have been arrived at on the basis of the steady-state optical and ESR experiments.

1. Red light illumination of solutions of chlorophyll or pheophytin (or other porphyrins) plus quinones or hydroquinones in ethanol or pyridine-water at temperatures above -50°C produces measurable quantities of only quinone anion radical species (as evidenced by optical and ESR experiments). These decay by a second order process (disproportionation).

2. At very low temperatures in ethanol (<-70°C), reversible chlorophyll bleaching is observed upon illumination along with positive absorbance changes in the 360-500 nm region. First order decay kinetics are observed.

3. In viscous alcoholic solvents (glycerol-ethanol and CBE) at room temperature and below, similar absorbance changes are observed as with ethanol at low temperatures. Decay kinetics are first order at all wavelengths. Rise kinetics are biphasic.
4. ESR signals generated in CBE with metal porphyrins are broad and structureless. These also decay by first order kinetics, but the decay constants are significantly faster than for the optical changes. Rise kinetics are also faster and are not biphasic.

5. The results mentioned in 3 and 4 show that at least two species are being generated by light; one of these is paramagnetic and one is not.

6. Initial rates of product formation are determined by the concentration of excited state species.

7. Adding acetic acid increases the magnitude of the steady-state concentration of photoproducts, decreases the decay rate and increases the initial velocities.

8. Adding small amounts of base (pyridine or potassium acetate) diminishes the magnitude of the signals. Larger amounts of base eliminate the chlorophyll bleaching and cause semiquinone anion radical formation by light.

9. Activation energies for chlorophyll-benzoquinone in CBE are 13-14 kcal/mole for all responses. This value decreases to 8-9 kcal/mole in ethanol-acetic acid. Trimethylbenzoquinone, a weaker oxidizing agent, gives a value of 2.5 kcal/mole in CBE.

10. Quenching of the photoreactions by \( O_2 \) and \( \beta \)-carotene demonstrates that the chlorophyll triplet state is the photoreactive species.
11. The nature of the porphyrin (particularly the presence or absence of a chelated metal ion and its identity) and quinone species affects both the magnitude of the various signals (ESR and optical) and the rise and decay kinetics. In general, the higher the redox potential of the quinone (or the more positive the half-wave potential), the slower is the decay and the larger are the signals. The results suggest that one of the intermediates being observed in these experiments is a complex containing both porphyrin and quinone species which interact via the central metal ion and in which one or both of the partners is paramagnetic, and that the decay process involves an electron transfer reaction occurring within the complex.
CHAPTER 5

FLASH PHOTOLYSIS EXPERIMENTS

In flash photolysis experiments, chlorophyll a by itself in CBE gives absorbance changes not unlike those obtained in steady illumination experiments in the presence of quinone (Fig. 94). Positive absorbance changes have been observed by us almost as far into the red as 800 nm. Decay kinetics are very sensitive to the presence of traces of oxygen. Fig. 95 is a superposition of three oscilloscope traces of the same sample on the first, sixth and thirteenth flashes showing a slowing down of the decay and a decrease in the maximum amplitude of the transient. After about thirteen flashes, the decay rate and amplitude remain constant. A thirteen percent decrease in the maximum absorbance change occurs between the first and the thirteenth flash. Presumably, the oxygen and some of the chlorophyll are being consumed in a photochemical process.

In the experiments shown below, decay curves were used only after an initial period of flashing was carried out.

*These effects are probably due to the difficulty of degassing the viscous CBE solvent, inasmuch as we do not observe this in ethanol or other more fluid media.
Figure 94. Flash photolysis difference spectrum for the system: chlorophyll a ($4.7 \times 10^{-6}$M) in CBE (refluxed) at rm. temp. $\Delta$ absorbance was taken 0.15 millisecond after the flash. These values are for a 5 cm cuvette.
Figure 95. Oscilloscope traces showing a comparison of decay kinetics for the first, sixth and thirteenth flashes at 460 nm, for the system: chlorophyll a (1.3 x 10^-5M) in CBE (refluxed) at rm. temp.
Decay kinetics are shown in oscilloscope traces for chlorophyll a in CBE and solvents such as tert-butanol, dimethylsulfoxide, chloroform, carbon tetrachloride and ethanol, for a few wavelengths (Figs. 96, 98, 100, 102, 104 and 106). Decay rates do not follow any simple kinetic order. Semi-log plots of the foregoing decay curves show that in most cases the rates are approximately the same at all wavelengths (Figs. 97, 99, 101, 103, 105 and 107). However, for the two most viscous solvents, CBE and DMSO, the decay of the bleaching of the Soret band (at 430 nm) appears to be faster. This may imply the presence of two or more species in these solvents which are either not obtained or not distinguished in the other solvents.

If some of the absorbance changes we observe in these systems are due to the chlorophyll triplet state, we should be able to demonstrate quenching by β-carotene. In Figs. 108-111 are shown the effects of the presence of β-carotene on the transients at 430, 470 and 660 nm in CBE. We observe marked increases in the rate of decay at 430 and 660 nm, whereas the transient at 470 nm is reduced slightly in magnitude with a much smaller increase in rate, even at considerably higher β-carotene concentrations. This shows that the absorbance changes at 430 and 660 nm reflect primarily the formation and decay of the chlorophyll triplet, while the changes at 470 nm result mainly
Figure 96. Oscilloscope traces showing decay kinetics after flash photolysis of the system: chlorophyll a (1.8 x 10^{-5}M) in GBE (refluxed) at rm. temp.

θ refers to baseline
Figure 97. First order decay plots for oscilloscope traces shown in Fig. 96.
Figure 98. Oscilloscope traces showing decay kinetics after flash photolysis of the system: chlorophyll a (7.1 x 10^{-6} M) in tert-butanol-isooctane (4:1) at rm. temp.
Figure 99. First order decay plots for oscilloscope traces shown in Fig. 98.
Figure 100. Oscilloscope traces showing decay kinetics after flash photolysis of the system: chlorophyll a (4.4 x 10^{-6}M) in dimethylsulfoxide at rm. temp.
Figure 101. First order decay plots for oscilloscope traces shown in Fig. 100.
Figure 102. Oscilloscope traces showing decay kinetics after flash photolysis of the system: chlorophyll $a$ ($4.5 \times 10^{-6}$M) in chloroform at rm. temp.
Figure 103. First order decay plots for oscilloscope traces shown in Fig. 102.
Figure 10. Oscilloscope traces showing decay kinetics after flash photolysis of the system: chlorophyll a (3.9 x 10^{-6}M) in carbon tetrachloride at rm. temp.
Figure 105. First order decay plots for oscilloscope traces shown in Fig. 104.
Figure 106. Oscilloscope traces showing decay kinetics after flash photolysis of the system: chlorophyll a (4.0 x 10^-6M) in ethanol at rm. temp.
Figure 107. First order decay plots for oscilloscope traces shown in Fig. 106.
Figure 108. Oscilloscope traces showing decay kinetics at 430 nm after flash photolysis for chlorophyll a (2.5 x 10^{-5}M) and chlorophyll a plus β-carotene in CBE (refluxed) at rm. temp.
Figure 109. First order decay plots for oscilloscope traces shown in Fig. 108.
Figure 110. Oscilloscope traces and first order decay plots showing decay kinetics at 470 nm after flash photolysis for the systems: chlorophyll a (4.7 x 10^-6M) and chlorophyll a plus β-carotene in CBE (refluxed) at rm. temp.
Figure III. Oscilloscope traces and first order decay plots showing decay kinetics at 660 nm after flash photolysis for the systems: chlorophyll a (2.4 x 10^-5 M) and chlorophyll a plus \( \beta \)-carotene in CBE (refluxed) at rm. temp.
from another species which is a product of a reaction of the triplet state, most probably with the solvent.

Different results are obtained in ethanol (Figs. 112 and 113). In this solvent, on adding β-carotene, about the same amount of rate increase is observed both at 520 nm and at 665 nm (520 nm gives results which parallel those at 470 nm). Thus, it would appear that the second photoproduct is generated to a much lesser extent, if at all, in ethanol than in CBE.

If one examines the flash decay curves of chlorophyll in CBE at slow sweep rates and higher amplification, one can observe a small slowly-decaying component (half-life around 40 millisec). This is shown in Figs. 114-116. Similar results are obtained at other wavelengths. We will discuss the interpretation of this in a later section.

Pheophytin also shows absorbance changes in CBE as measured by flash spectroscopy. Both positive and negative absorbance changes are obtained as with chlorophyll (Fig. 117). The decay rates at 470 nm for both chlorophyll and pheophytin are roughly the same, but the decay of the Soret band (at 410 nm) appears to be four times faster than the decay of the chlorophyll Soret. Decay rates at 470 and 660 nm are roughly the same (see oscilloscope traces in Fig. 118 and first order plots in Fig. 119). Therefore, qualitatively we have the same situation as with chlorophyll.
Figure 112. Oscilloscope traces and first order decay plots showing decay kinetics at 520 nm after flash photolysis for the systems: chlorophyll a (4.5 x 10^-6M) and chlorophyll a plus b-carotene in degassed ethanol at rm. temp.
Figure 113. Oscilloscope traces and first order decay plots showing decay kinetics at 665 nm after flash photolysis for the systems: chlorophyll a (4.5 x 10^{-6}M) and chlorophyll a plus β-carotene in degassed ethanol at rm. temp.
Figure 114. Oscilloscope traces at 2 and 5 millisecond full scale sweep showing decay kinetics at 460 nm for the system: chlorophyll a \( (1.1 \times 10^{-5}\text{M}) \) in CBE (refluxed) at rm. temp.
Figure 115. Oscilloscope traces at 10 and 50 millisec full scale sweep showing decay kinetics at 460 nm for the system: chlorophyll a (1.1 x 10^-5M) in CBE (refluxed) at rm. temp.
Figure 116. Oscilloscope traces at 100 and 1000 millisec full scale sweep showing decay kinetics at 460 nm for the system: chlorophyll a \((1.1 \times 10^{-5} \text{M})\) in CBE (refluxed) at rm. temp.
Figure 117. Flash photolysis difference spectrum for the system: pheophytin a (5 x 10^{-6}M) in CBE (refluxed) at rm. temp. These $\Delta$absorbanes are for a 5 cm cuvette and were taken 0.2 millisecond after the flash.
Figure 118. Oscilloscope traces showing decay kinetics after flash photolysis for the system: pheophytin a (5 x 10^-6M) in CBE (refluxed) at rm. temp.
Figure 119. First order decay plots (log $\Delta$ absorbance versus time) for oscilloscope traces shown in Fig. 118.
Further similarities with chlorophyll are seen in the effects of β-carotene. At 470 nm little or no difference is observed in the decay rates on addition of β-carotene up to as much as $2 \times 10^{-3}$M (Fig. 120). However, at the Soret band (410 nm) the decay is speeded up with $7.5 \times 10^{-4}$M β-carotene (Fig. 121).

**Flash Experiments in the Presence of Quinones**

The addition of quinones to solutions of chlorophyll in CBE results in slowly-decaying species being generated during flash excitation. This is shown for benzoquinone by the traces in Fig. 122. Three decay processes can be clearly distinguished in all wavelength regions in these flash experiments (Figs. 122, 123 and 124). One is very fast, with a half-life of about 0.2 millisecond. This probably corresponds to triplet state decay (compare with trace of chlorophyll alone in Fig. 122). The second component (quantitatively the largest) has a half-life of 5 to 10 milliseconds. This decay time seems to be independent of quinone concentration (see traces, Fig. 125 and first order plots, Fig. 126). The third (generally smaller) component plots as a first order decay and has a half-life of several seconds which is quite clearly a function of quinone concentration, becoming faster at higher concentrations (Fig. 127). This dependency is probably the consequence of
Figure 120. Oscilloscope traces and first order decay plots showing decay kinetics at 470 nm after flash photolysis for the systems: pheophytin a (5 x 10^{-6} M) and pheophytin a plus β-carotene in CBE (refluxed) at rm. temp.
Figure 121. Oscilloscope traces and first order decay plots showing decay kinetics at 410 nm after flash photolysis for the systems: pheophytin a (5 x 10^{-6}M) and pheophytin a plus β-carotene in CBE (refluxed) at rm. temp.
Figure 12. Oscilloscope traces at 470 nm (top) and 650 nm (bottom) showing decay kinetics after flash photolysis for the systems: chlorophyll a and chlorophyll a plus p-benzoquinone in CBE (refluxed) at rm. temp.
Figure 123. Oscilloscope traces at 5 and 50 millisec full scale sweep times showing decay kinetics at 460 nm for the system: chlorophyll a (1.2 x 10^-5M), p-benzoquinone (2.0 x 10^-4M) in CBE (refluxed) at rm. temp.
Figure 124. Oscilloscope traces at 10 and 50 sec full scale sweep times showing decay kinetics at 460 nm for the system: chlorophyll a ($1.2 \times 10^{-5}$M), p-benzoquinone ($2.0 \times 10^{-4}$M) in CBE (refluxed) at rm. temp.
Figure 125. Oscilloscope traces showing a comparison of decay kinetics after flash photolysis at 460 nm for various concentrations of p-benzoquinone for the systems: chlorophyll a (1.2 x 10^{-5}M), p-benzoquinone (various concentrations) in GBE (refluxed) at rm. temp.
Figure 126. First order decay plots for oscilloscope traces shown in Fig. 125.
Figure 127. Recorder traces showing a comparison of decay kinetics at 460 nm (top) and 620 nm (bottom) after flash photolysis for various concentrations of p-benzoquinone for the systems: chlorophyll a (1.6 x 10^{-5}M), p-benzoquinone in CBE (refluxed) at rm. temp.
a pseudo-first order process due to the presence of excess quinone. This can be seen by the linear plots of decay constants versus quinone concentration (Fig. 128). Note that the decay at 460 nm is more sensitive to quinone concentration than is the decay at 620 nm.

The fast initial decay is usually difficult to observe in these systems. The first slow decay process (half-life of 3 to 7 millisec) is relatively independent of quinone species, with the exception of p-naphthoquinone (Figs. 129-132). In the case of naphthoquinone, the potential may be too low to elicit a sizeable amount of the species responsible for the slow decays. That naphthoquinone has little effect on the decay of chlorophyll absorbance changes (in this time region) is seen in the first order decay plots (Figs. 130 and 132). The decay rates of the slowest processes are definitely dependent on quinone species. They are slower for higher potential quinones and faster for lower potential quinones (Fig. 133). This is similar to what was observed in the steady-state experiments (see above).

Quinone potential is also a factor in the divergence of decay rates between the 460 nm band and the red band in some flash experiments (see Figs. 127 and 128). In a flash experiment using different quinones at equal concentrations (quinone to chlorophyll ratio of 5.8), it was found that
Figure 128. Plots of the first order decay constants for the slowest flash decay versus benzoquinone concentration. System: chlorophyll a (1.6 x 10^-3M), p-benzoquinone (various concentrations) in CBE (refluxed) at rm. temp.
Figure 129. Oscilloscope traces showing a comparison of decay kinetics after flash photolysis at 460 nm for various quinones for the systems: chlorophyll a (1.6 x 10^{-5}M), quinones, in CBE (refluxed) at rm. temp.
Figure 130. First order decay plots for oscilloscope traces shown in Fig. 129 plus the first order decay plot of chlorophyll a alone in CBE at 460 nm.
Figure 131. Oscilloscope traces showing a comparison of decay kinetics after flash photolysis at 620 nm for various quinones for the systems: chlorophyll a (1.6 x 10^{-5} M), quinones, in CBE (refluxed) at rm. temp.

- Plus 0.93 x 10^{-4} M methyl-p-benzoquinone
- Plus 0.93 x 10^{-4} M dimethyl-p-benzoquinone
- Plus 0.93 x 10^{-4} M trimethyl-p-benzoquinone
- Plus 0.93 x 10^{-4} M p-naphthoquinone
Figure 132. First order decay plots for oscilloscope traces shown in Fig. 131 plus the first order decay plot of chlorophyll a alone in CBE at 620 nm.
for quinones whose potential is less than that of benzo-
quinone the decay rates were about equal (Fig. 134).

If one compares the slowest decay observed in the
flash photolysis experiments (red band bleaching), the
steady-state ESR experiments and the steady-state optical
experiments, one finds that the steady-state optical decay
is much slower than the other two (Fig. 135). The differ­
ence in decay rate observed between the steady-state ESR
signal and the slowest flash decay depends on the molar
ratio of chlorophyll to quinone. If the concentrations
are about equal, then the two decay rates are approximately
equal. With increasing quinone, the ESR decay possibly
becomes slightly slower, and the slowest flash decay be­
comes faster (Fig. 136).

Thus, the presence of excess quinone causes changes
in decay rates under both flash (see also Fig. 128) and
steady illumination conditions. In the case of steady il­
lumination we may be observing the formation of some
slower-decaying species. We will deal with this again in
the discussion section.

Quite different flash behavior is obtained in etha­
nol and tert-butanol upon addition of quinone. In these
solvents, one observes a decrease in the size of signal at
all wavelengths (Figs. 137 and 138). Decay kinetics (in
ethanol) are not affected at 460 and 665 nm (Figs. 139 and
140), although they seem to be accelerated at 430 nm
Figure 133. Recorder traces showing a comparison of decay kinetics at 460 nm (top) and 620 nm (bottom) after flash photolysis for various quinones for the systems: chlorophyll a (1.6 x 10^{-5}M), quinones, in CBE (refluxed) at rm. temp.
Figure 13. Plot of the ratio of the 460 nm first order decay constant to the 620 nm first order decay constant ($k_{460}/k_{620}$) versus quinone redox potential for slowest flash decay (measured by strip-chart recorder). System: chlorophyll a ($1.6 \times 10^{-5}$M), quinone ($0.93 \times 10^{-4}$M) in CBE (refluxed) at rm. temp.
Figure 135. Kinetic curves for ESR and optical experiments for the system: chlorophyll a \((0.9 \times 10^{-5} \text{M})\), p-benzoquinone \((1.3 \times 10^{-5} \text{M})\) in CBE (refluxed) at rm. temp. (a) Steady-state ESR signal rise and decay. (b) Steady-state optical bleaching rise and decay at 430 nm. (c) Flash optical decay at 650 nm.
Figure 136. Plots of the log of the first order flash decay constant for bleaching at 620 nm (slowest decay from strip-chart recorder) and the log of the first order steady-state ESR decay constant versus the molar ratio of benzoquinone to chlorophyll a (BQ/Chl). System: chlorophyll a, benzoquinone, in CBE (refluxed) at rm. temp.
Figure 137. Oscilloscope traces showing decay kinetics at 460 nm (top) and 620 nm (bottom) after flash photolysis for chlorophyll a (2 x 10^-5 M) and chlorophyll a plus p-benzoquinone in degassed tert-butanol-isooctane (4:1) at rm. temp.
Figure 138. Oscilloscope traces showing decay kinetics at 430 nm (top), 460 nm (center) and 665 nm (bottom) after flash photolysis for chlorophyll a ($4 \times 10^{-6}$M) and chlorophyll a plus $7.6 \times 10^{-4}$M p-benzoquinone in degassed ethanol at rm. temp.
Figure 139. First order decay plots of oscilloscope traces shown in Fig. 138 (center) for decay kinetics at 460 nm after flash photolysis, for chlorophyll a ($4 \times 10^{-6}M$) and chlorophyll a plus p-benzoquinone in degassed ethanol at rm. temp.
Figure 140. First order decay plots of oscilloscope traces shown in Fig. 138 (bottom) for decay kinetics at 665 nm after flash photolysis, for chlorophyll a (4 x 10^{-6}M) and chlorophyll a plus 7.6 x 10^{-4}M p-benzoquinone in degassed ethanol at room temp.
(Fig. 14). This behavior parallels that observed in pyridine (Raman and Tollin 1970) and so we may assume that the nature of the reaction is the same (see below). It is worth recalling here that, in these solvents, one obtains no chlorophyll bleaching in steady-state experiments and the paramagnetic species generated is always the semiquinone anion radical.

Addition of quinone to pheophytin solutions in CBE produces similar quenching of the signal size as with chlorophyll in ethanol or tert-butanol (Fig. 142). No slowly decaying species are generated. This is consistent with our observations of no steady-state photobleaching of pheophytin and formation of semiquinone radical species.
Figure 141. First order decay plots of oscilloscope traces shown in Fig. 138 (top) for decay kinetics at 430 nm after flash photolysis, for chlorophyll a (4 x 10^{-6}M) and chlorophyll a plus p-benzoquinone in degassed ethanol at rm. temp.
Figure 142. Oscilloscope traces showing decay kinetics at 410 nm (top) and 460 nm (bottom) after flash photolysis for pheophytin a (5 x 10^{-5}M) and pheophytin a plus p-benzoquinone in CBE (refluxed) at rm. temp.
Several phenomena occurring during steady illumination of viscous alcoholic solutions of chlorophyll and quinone point toward the formation of a paramagnetic chlorophyll-quinone complex:

1. The type of ESR spectrum (due to free semiquinone) which is observed in ethanol and pyridine is not observed in CBE. The broad single-line spectrum with no hyperfine structure which is obtained could be accounted for by spin dipole-dipole interaction within a biradical complex (Hedvig and Zentai 1969) or by a slow tumbling rate* and a large number of hyperfine interactions in a monoradical complex in which the unpaired electron is delocalized over both species.

2. The first order decay kinetics observed in both optical and ESR experiments are consistent with either an internal electron transfer:

\[ \text{Chl}^{+} \ldots \text{Q}^{-} \rightarrow \text{Chl} + \text{Q} \]

---

*The solvent viscosity is not the determining factor here inasmuch as we observe hyperfine structure with pheophytin-quinone in CBE.
or a slow dissociation of a monoradical complex followed by rapid disproportionation:

\[(\text{Chl} \cdots Q^-) \rightarrow \text{Chl} + Q^-\]

The formation of this complex is dependent upon the basicity of the solvent. Adding small amounts of acetic acid increases the amount of this intermediate, as long as the pH does not decrease to the point of pheophytinization. Below a pH of about 3, light will induce an irreversible loss of magnesium ion. Above this pH a light-induced reversible pheophytinization can occur (Rabinowitch 1945, p. 493). This latter process is probably the cause of the very slowly decaying absorbance changes which we observe in steady-state experiments in the absence of quinone. It is very suggestive that the presence of quinone seems to stabilize the chelation of magnesium, as evidenced by the disappearance of these very slow changes.*

*Evstigneev and Gavrilova (1968) suggested that the inhibition of pheophytinization by quinone during illumination of a degassed ethanol solution of chlorophyll and quinone is due to a rise in pH. According to this interpretation, the pH change is a result of the initial formation of the semiquinone anion radical which then acts as a base. This seems unlikely in view of the ESR evidence for the semiquinone anion radical in the steady-state and observations of pH decreases in such systems (Quinlan and Fujimori 1967). Thus, one could postulate a stabilization resulting from coordination of quinone by the magnesium (see below).
If CBE is made basic (by the addition of a small amount of solid potassium acetate or pyridine), only the formation of the semiquinone anion radical is detected. In this case, as in the case of ethanol, the light-off decay kinetics are second order in spin concentration. These results indicate that the viscosity of the solvent is not the determining factor in the difference in behavior between CBE and ethanol (see below).

3. If instead of chlorophyll, pheophytin (chlorophyll without chelated magnesium) is used in CBE, no absorbance changes in the red spectral region can be observed and no slowly decaying species are formed upon quinone addition. The lack of chlorophyll bleaching has been noted before by Russian workers (Evstigneev and Gavrilova 1950, 1954). However, ESR experiments (Fig. 81) do show the formation of benzosemiquinone radical (the monoprotonated species). Thus, the presence of the chelated metal ion is necessary in order to observe light-induced changes in the porphyrin optical spectrum and the broad unstructured ESR signal with its slow first order decay.

We have found that hematoporphyrin behaves like pheophytin and that zinc and cadmium chelates of pheophytin and hematoporphyrin behave similarly to chlorophyll, although transition metal chelates such as those of Ni and Co (with unpaired electrons in d-orbitals) do not. This latter
effect may be due to paramagnetic triplet quenching. It is interesting to note that Rennert et al. (1969) have found that group II metal ions complex and stabilize the semi-quinone radical of phenanthroquinone in degassed ethanol.

These results suggest that the paramagnetic complex of chlorophyll and quinone is formed through the chelated metal. This allows a simple explanation for the solvent dependence of complex formation. Thus, one need only suppose a competition between solvent and quinone for coordination with the metal ion. In ethanol, pyridine or CBE in the presence of bases such as acetate or pyridine, the quinone plus the unpaired electron is displaced by solvent and one observes only the semiquinone radical. In CBE by itself, the quinone is not displaced and one observes the complex. That this explanation is reasonable is shown by the fact that small amounts of base added to CBE decrease the amount of ESR signal and increase its rate of decay. A further factor in the case of CBE is its acidity. This is shown by the fact that the semiquinone radical exists as the protonated species in this solvent. We will discuss the possible basis for the effect of acid below.

We must now inquire into the mechanism of formation and the nature of the chlorophyll-quinone complex. Two possibilities exist:
1. Reaction of chlorophyll triplet with quinone to give an oxidized chlorophyll-quinone (biradical) complex:

\[ \text{Chl}_t + Q \rightarrow \text{(Chl}^\text{ox}_\text{ox} \ldots Q^\text{7}) \]

2. Reaction of chlorophyll triplet with solvent to give reduced chlorophyll and oxidized solvent, with a rapid reaction of reduced chlorophyll and quinone to form a complex:

\[ \text{Chl}_t + \text{Solv} \rightarrow \text{ChlH}^\text{•} + \text{Solv}^\text{ox} \]

\[ \text{ChlH}^\text{•} + Q \rightarrow \text{(Chl} \ldots Q^\text{H})^\text{•} \]

Several lines of evidence favor the second of these possibilities:

1. No ESR signals for a free chlorophyll radical have ever been observed in these systems. Dissociation of a biradical complex would give equal amounts of \( Q^\text{7} \) and \( \text{Chl}^\text{•ox} \), whereas dissociation of a reduced chlorophyll complex would form \( Q^\text{7} \) and ordinary chlorophyll.

2. Semiquinone radical decay in ethanol is by disproportionation rather than reverse electron transfer. This would be expected on the basis of mechanism 2, if one assumes that the oxidized solvent radical is very unstable and dimerizes (or disproportionates) quickly, leaving the

*This can also be formulated as a photoinduced electron transfer within a ternary complex of solvent, chlorophyll and quinone (see below).
quinone radical free to disproportionate.

The simplest reaction of chlorophyll triplet with an alcoholic solvent would be hydrogen abstraction (inasmuch as a similar photoproduct is obtained in tert-butanol, the hydroxyl hydrogen would seem to be involved):

\[
\text{Chl} + \text{hv} \rightarrow \text{Chl}_s \rightarrow \text{Chl}_t \xrightarrow{k_1} \text{Chl} \ldots (1)
\]

\[
\text{Chl}_t + \text{ROH} \rightarrow \text{ChlH}^\cdot + \text{RO}^\cdot \ldots \ldots (2)
\]

\(k_1\) Represents all modes of decay of the triplet state.

Triplet quenching by \(\beta\)-carotene would lead to an increase in triplet decay rate and a decrease in the amount of \(\text{ChlH}^\cdot\) formed. This could account for the difference in behavior of the transients at 430 nm, 460 nm and 660 nm in the presence of this quencher* (Figs. 108-111). Depending on the basicity of the solvent, the reduced chlorophyll radical could dissociate:

\[
\text{ChlH}^\cdot \rightarrow \text{Chl}^- + \text{H}^+ \ldots \ldots (3)
\]

It is not possible to unequivocally decide which form of chlorophyll radical is important in the present studies.

*It is interesting that \(\beta\)-carotene in hydrocarbon solvents, in ethanol or in pyridine quenches chlorophyll transients at all wavelengths with approximately equal effectiveness (Figs. 112 113 and Raman and Tollin 1970). This suggests that in these solvents, little or no hydrogen abstraction by excited chlorophyll occurs in the absence of quinone.
However, the effect of adding acetic acid to CBE solutions suggests that the neutral radical may be the most significant in quinone reactions, i.e. that the chlorophyll-quinone complex is formed only with this species (see below).

Because of the overall reversibility of the photo-reaction, we propose that the reverse hydrogen transfer reaction is fast:

\[
\text{ChlH} + \text{RO} \cdot \xrightarrow{k_3} \text{Chl} + \text{ROH} \quad \ldots \ldots \quad (4)
\]

However, an alkoxyl radical should also be capable of dimerization, which would compete with reaction (4):

\[
2\text{RO} \cdot \xrightarrow{k_4} \text{ROOR} \quad \ldots \ldots \ldots \ldots \ldots \ldots \quad (5)
\]

Inasmuch as the peroxide so generated is an oxidizing agent, it could also function to oxidize ChlH•:

\[
\text{ChlH}^* + \text{ROOR} \xrightarrow{k_5} \text{Chl} + \text{ROH} + \text{RO} \cdot \ldots \ldots \quad (6)
\]

Equations (4), (5) and (6) account for the lack of simple kinetics at 460 nm, and can also explain the very slow decay process observed in flash experiments in CBE (Figs. 114, 115 and 116).

Reactions (1)-(6) are consistent with all of the flash photolysis results obtained in the absence of quinones. When quinones are present in ethanol or tert-butanol, one
observes a decrease in the amount of photoprocess with little or no change in decay rates (at least at 460 and 660 nm) in a flash experiment and the rapid formation of semiquinone anion radical (see Fig. 25). This is the same type of behavior observed by Raman and Tollin (1970) in pyridine, and thus the mechanism proposed for this solvent probably also applies to these alcohols. This involves a photochemical reaction* proceeding within a ternary complex of solvent, chlorophyll and quinone:

\[
\text{(ROH---Chl---Q) + h\nu \rightarrow RO^- + Chl} \\
+ \text{QH}^- . \quad (7)
\]

The increase in decay rate at 430 nm (Fig. 141) may reflect the disappearance of some of the quinone radical via reaction with oxidized solvent.

Reaction (7) can easily explain all of our steady-state ESR and optical results at room temperature (no chlorophyll bleaching or radical signals, semiquinone anion radical formation).

The quinone radical may also disproportionate (this was demonstrated by our earlier ESR experiments):

*It is also known that quinone quenches Chl$_g$ and Chl$_t$ (Raman and Tollin 1970). However, since the chlorophyll bleaching decreases in the presence of quinone, these are probably not photochemical processes.
Regeneration of $Q$ can proceed via the following reactions, both of which would be expected to be rapid:

$$2Q^- + 2H^+ \xrightarrow{k_7} Q + H_2Q \ldots \ldots \ldots \ldots (8)$$

$$H_2Q + RO \rightarrow ROH + Q^- + H^+ \ldots \ldots \ldots (9)$$

$$H_2Q + ROOR \rightarrow 2ROH + Q \ldots \ldots \ldots (10)$$

This accounts for the complete reversibility of the quinone-chlorophyll photosystems.

As we have shown, a paramagnetic chlorophyll-quinone complex is stabilized in CBB (or in ethanol at low temperature) via coordination with the central metal ion. In such a complex, the unpaired electron would probably delocalize throughout the porphyrin and quinone pi systems. The kinetic stability of the complex must be related to the extent of such delocalization, since we observe that the higher the quinone potential (which reflects a higher electron affinity) the slower is the decay. This would suggest that it is the quinone radical which is the principal form in the complex rather than the oxidized form. If this is so, then the effect of acid in increasing the amount of complex formed could be due to an increase in the concentration of the neutral chlorophyll radical (equation 3) which forms a stronger complex because of electrostatic attraction between the positive chlorophyll and the negative quinone radical:
When the chelated metal is varied, we observe changes in decay rates, in the order \( \text{Cd}^{+2} > \text{Zn}^{+2} > \text{Mg}^{+2} \) (Table 11). This ordering of rates is the same as the order of the ease of reduction of the metal ion. Thus, again there is a correlation between electron delocalization onto the quinone and complex stability. A further observation which is pertinent here is that zinc hematoporphyrin gives slower decay kinetics than does zinc pheophytin (Table 11). Inasmuch as the former has a more extensive pi electron system, this is consistent with a relation between ease of electron delocalization and kinetic stability.

In attempting to formulate the reactions which are occurring in CBE, we must keep in mind the following additional facts:

1. Two decay processes are observed in the flash experiments, one relatively fast and quinone concentration and species independent and the other slow (although faster than the ESR and steady-state optical decays at higher quinone concentrations) and increasing in rate with increasing quinone concentration and decreasing potential (with a difference of magnitude between 460 and 660 nm for high potential quinones).

2. The rise kinetics for the steady-state optical signals are complex and are faster in the initial portion at
Table 11. First order light-off decay constants and percent $\Delta$ absorbance for steady-state bleaching for several metal-porphyrins and quinones. Systems: metal-porphyrin (about $10^{-5}M$), quinone ($10^{-2}M$) in CBE (refluxed) at rm. temp.

<table>
<thead>
<tr>
<th>Quinone</th>
<th>Dichloro-benzoquinone</th>
<th>Benzoquinone</th>
<th>Methyl-benzoquinone</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chlorophyll a</strong></td>
<td>k (sec$^{-1}$)</td>
<td>$% \Delta A$</td>
<td>k (sec$^{-1}$)</td>
</tr>
<tr>
<td>(Mg-pheophytin a)</td>
<td>0.0049 ± 0.0003</td>
<td>2.8 ± 0.1</td>
<td>0.014 ± 0.1</td>
</tr>
<tr>
<td><strong>Bacteriochlorophyll</strong></td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>Zn-Pheophytin a</strong></td>
<td>0.041 ± 0.003</td>
<td>0.83 ± 0.00</td>
<td>0.25 ± 0.03</td>
</tr>
<tr>
<td><strong>Cd-Pheophytin</strong></td>
<td>0.064 ± 0.008</td>
<td>0.67 ± 0.10</td>
<td>0.52 ± 0.07</td>
</tr>
<tr>
<td><strong>Zn-Hematoporphyrin</strong></td>
<td>0.0050 ± 0.0001</td>
<td>2.3 ± 0.1</td>
<td>0.0092 ± 0.0006</td>
</tr>
<tr>
<td><strong>Zn-Pthalocyanine</strong></td>
<td>--</td>
<td>--</td>
<td>0.0082 ± 0.0005</td>
</tr>
</tbody>
</table>
460 nm and 430 nm than at 660 nm. Only the rapidly-produced species is paramagnetic. The decay kinetics of the optical signals are the same at all wavelengths and are the slowest of all.

The initial, rapid formation of a slowly-decaying species (occurring within the flash lifetime) is undoubtedly the following (by analogy with the pyridine and ethanol results):

\[
(\text{ROH}--\text{Chl}--\text{Q}) + h\nu \longrightarrow (\text{ChlH}^+---\text{Q}^-) + \text{RO}^* \ldots \ldots \text{(11)}
\]

Such a complex would be unstable and its decay should occur by dissociation (the electron leaving with the quinone):

\[
(\text{ChlH}^+---\text{Q}^-) \longrightarrow \text{Chl} + \text{QH}^* \ldots \ldots \ldots \ldots \text{(12)}
\]

Reaction (12) could correspond to the intermediate, quinone concentration independent decay process observed in the flash experiments (the quinone radical decaying by disproportionation, as before). We propose that the electron remains with the quinone so as to be consistent with reaction (7). This is also to be expected on chemical grounds.

---

*It is difficult to rationalize why such a dissociation should be relatively independent of quinone potential unless the unpaired electron were almost completely localized on the quinone.
At the high levels of quinone used in these experiments, it is possible that the 1:1 complex reacts with another quinone molecule to form a 1:2 complex:

$$(\text{ChlH}^+\text{--Q}^-) + \text{Q} \rightarrow (\text{Q}--\text{ChlH}^+\text{--Q}^-)$$  \hspace{1cm} (13)

This would again decay by a similar dissociation:

$$(\text{Q}--\text{ChlH}^+\text{--Q}^-) \rightarrow \text{Chl} + \text{Q} + \text{QH}^- \hspace{1cm} (14)$$

However, the additional quinone molecule would further delocalize the unpaired electron and, in terms of our previous arguments, would probably make reaction (14) intrinsically slower than reaction (12). We suggest that the 1:2 complex is what is being observed in the ESR experiments, which are generally carried out at high quinone concentrations. At low quinone concentration, the ESR and flash decay rates approach each other, suggesting that both methods are observing the same species (1:1 complex). The increase in decay rate of the ESR signal at low quinone concentration (Fig. 136) is consistent with this. Reaction (14) also accounts for the first order nature of the ESR decay. The additional quinone molecule, by further decreasing the electron density on the chlorophyll, and perhaps also partially removing the proton by hydrogen bonding, might tend to make the absorption spectrum of this species more similar to that of ordinary chlorophyll than
would be the case for the 1:1 complex. Thus, the quinone concentration and quinone species dependent absorbance decrease observed in the flash experiment could be reaction (13). This can also account for the differing decay rates seen at 460 nm and 660 nm in the flash results with benzo-quinone (Fig. 128). Thus, if the 1:2 complex has lost much of the 460 nm absorbance, while still retaining some bleaching in the red, the slower rate of reaction (14) would tend to compensate at 660 nm but not at 460 nm for the increase in decay rate caused by reaction (13). Lower potential quinones would form less stable 1:2 complexes, thus causing reaction (14) to become rapid (Fig. 133). This would tend to make the differences at 460 nm and 660 nm less apparent, as is observed (Fig. 134).

One would not expect much of the 1:2 complex to be formed in a flash experiment due to the instability of the 1:1 complex and the bimolecular nature of its formation. Thus, a separate decay process reflecting reaction (14) might be difficult to observe. On the other hand, this species would tend to build up in a steady-state experiment such as ESR.

*This is perhaps not unreasonable, inasmuch as the unpaired spin density on the chlorophyll should be quite low but there should still be an appreciable perturbation of the chlorophyll absorption spectrum by the complexed quinone molecules.
The biphasic rise kinetics observed in the optical experiments can be explained in terms of a photoproduct interacting with the 1:2 complex. This is most likely ChlH* (although it could also be another molecule of the 1:1 complex):

\[
\text{ChlH*} + (\text{Q\text{-dimer}ChlH}^+\text{-Q}^-) \rightarrow (\text{ChlH}^+\text{-Q}^-\text{-ChlH}^+\text{-Q}^-)
\]

\[
(\text{ChlH}_2\text{-Q}^-\text{-Chl}\text{-Q})
\]

\[
(\text{Chl}\text{-QH}_2\text{-Q})
\]

(15)

If appreciable electron pairing occurs, this species* would be diamagnetic. This is in agreement with our results. Also, if the ChlH\(_2\) form is important, one might expect the absorbance in the red region to be smaller than in the 1:2 complex. This would explain the very slow bleaching process at 660 nm observed in the steady-state optical experiments. The rate of formation of this species should depend on the nature of the quinone used, in agreement with our results. The biphasic nature of the rise

*It is also possible to formulate this as follows:

\[(\text{ChlH}^\ast\text{-QH}^\ast) \leftrightarrow (\text{ChlH}_2\text{-Q})\]
curve can be accounted for by the intermediacy of the 1:2 complex. The fact that reaction (15) produces a diamagnetic species explains the monophasic nature of the ESR rise curve.

Such a 2:2 complex can again decay by a first order dissociation:

\[(2:2 \text{ complex}) \rightarrow 2\text{Chl} + Q + QH_2 \quad \ldots \ldots (16)\]

The extensive electron delocalization and lack of radical character in such a species should result in appreciable stability, thus accounting for the very slow decay and the build-up of this product upon prolonged illumination. This also explains the differences observed between the ESR and optical decays.

Inasmuch as all of the species which we are observing (the 1:1 complex which is the predominant form seen in the flash experiments, the 1:2 complex which is the predominant ESR active product and the 2:2 complex which produces the largest steady-state optical signals) contain both porphyrin and quinone moieties, we would expect their decay rates and activation energies to respond to changes in porphyrin and quinone structure in similar ways. This is in agreement with our observations.

The above scheme, while perhaps not unique, is probably the simplest which can successfully account for all of our results. Even if all of the details of
interpretation are not entirely correct, a clear and very interesting feature of these experiments is the apparent ability of relatively small changes in solvent environment (e.g. ethanol vs. CBE) to cause interactions which stabilize otherwise very highly reactive species (ChlH• or ChlH+, Q•-, ChlH2). Such effects could have important implications for the photosynthetic system, in which such reactive entities must have long enough lifetimes to effectively participate in energy-conserving redox reactions. Furthermore, the formation of alkoxy radicals from alcohols (and peroxides by dimerization) provides a simple chemical model for photosynthetic oxygen production.
REFERENCES


