The Effect of Ultraviolet Light on Mitochondria

II. RESTORATION OF OXIDATIVE PHOSPHORYLATION WITH VITAMIN K₁ AFTER NEAR-ULTRAVIOLET TREATMENT*

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The demonstration by Martius and Nitz-Litzow (4), and Martius (5), that vitamin K₁ may play a functional role in oxidative phosphorylation in animals has led to an increased interest in the participation of vitamin K₁ and related quinones in electron transport, oxidative phosphorylation, and photosynthetic phosphorylation. Since vitamin K₁ is altered by the action of ultraviolet radiation (6), we have treated rat liver mitochondria with near-ultraviolet light in order to alter such intramitochondrial components. After such treatment, oxidative phosphorylation with succinate as substrate is dependent upon the addition of cytochrome c, and oxidative phosphorylation with glutamate as substrate is dependent upon the addition of cytochrome c and vitamin K₁. The action of vitamin K₁ appears to be specific in that a number of related compounds do not restore oxidative phosphorylation.

EXPERIMENTAL

Reagents—Common chemicals were of analytical reagent grade. Solutions were prepared in deionized water and adjusted to pH 7.4. The various coenzymes were procured from the Sigma Chemical Company; vitamin K₁ was purchased from California Foundation for Biochemical Research; and d,а-tocopherol from Distillation Products Industries.

Mitochondrial Preparation—Rat liver mitochondria, washed twice, were prepared in 0.25 M sucrose essentially according to Schneider and Hogeboom (7). Homogenization was performed in a motor-driven glass homogenizer with a Teflon pestle. All operations before final incubation with substrate were carried out at 0-2°.

Irradiation Procedure—Mitochondria, at a density of 167 mg. equivalents per ml. (mitochondria derived from 167 mg. of fresh rat liver in 1 ml.) were irradiated with 14 General Electric, 18-inch BLB lamps¹ for 60 minutes in a rotary irradiator previously described (8). Quartz tubes were employed for the mitochondrial irradiation and control mitochondria were treated identically except that the tubes were covered with aluminum foil during irradiation. After irradiation, the particles were resuspended by high speed centrifugation.

¹ This work was done under the terms of Contract AT(30-1)-911 between the Physiology Department, Tufts University School of Medicine, and the Atomic Energy Commission. A preliminary communication has appeared (1) and was presented in part before the American Society of Biological Chemists, Philadelphia, Pennsylvania, April 17, 1958 (2). Part I (3) of this series concerns the effect of 2537 A light.

Over 93 per cent of the emission energy of these lamps is between 3200 and 3800 A with maximal emission at 3550 A.
measured by the conventional Warburg manometric technique, readings being obtained every 5 minutes for a period of 20 minutes. Since no equilibration period was employed, oxygen consumption for the first 5 minutes was obtained by extrapolation. This would tend to produce P:O ratios slightly lower than those which obtain when an equilibration period is used since phosphorylation probably proceeds more slowly during the first 5 minutes (during temperature elevation of the chilled mitochondrial suspension) than during subsequent periods and oxygen consumption for the first 5 minutes is taken as equal to the second 5-minute period.

Photochemical Breakdown of Vitamin K₁—The alteration of the vitamin K₁ molecule catalyzed by near-ultraviolet light was determined by irradiating absolute ethanol solutions of vitamin K₁ in quartz Beckman cuvettes placed 1 cm. from the surface of one General Electric 18-inch BLB lamp. Extinction loss was measured with a Beckman model DU spectrophotometer for 1 hour at 3280 and 3660 A after each 10 minutes of irradiation.

RESULTS

Irradiation Effect—When mitochondria were irradiated with near-ultraviolet light for varying periods of time, a loss of both the oxidative and phosphorylative ability with glutamate and succinate as substrates was observed (Table I). The data in Table I also reveal that oxidation and phosphorylation with glutamate as substrate were more sensitive to the radiation than were those from succinate. Also apparent was the greater sensitivity to irradiation of the phosphorylative system linked to both substrates, as compared to the oxidative system.

Restoration with Vitamin K₁—Inactivation of oxidative phosphorylation with succinate as substrate was partially reversed by the inclusion of cytochrome c to the mitochondrial system (Table II). Lower concentrations of cytochrome c than that reported did not appear to be as effective, and greater concentrations were at times somewhat less effective restorers of high P:O ratios. Vitamin K₁ plus cytochrome c did not significantly inhibit the control mitochondrial system with either succinate or glutamate as substrate. The addition of vitamin K₁ to the irradiated system with succinate as substrate had no effect on the P:O ratios when the system was either inhibited by near-ultraviolet treatment or restored by the addition of cytochrome c. Albumin was without restorative effect when either substrate was employed. With glutamate as substrate the addition of cytochrome c, DPN, or albumin did not result in restoration. The addition of vitamin K₁ alone partially restored the oxidation of glutamate and associated phosphorylations, the latter effect being of greater magnitude. Inclusion of cytochrome c plus vitamin K₁ restored both glutamate oxidation and electron transport phosphorylation to nearly original levels, resulting in nearly maximal P:O values.

Effect of Other Quinones—A number of other preparations were tested for restorative activity (Table III). Of those listed in Table III, only three appeared to have any influence on the radiation-inhibited system. Vitamin K₁ exhibited its usual restorative effect on oxidation and phosphorylation. Both vitamin K₁ diacetate and the Mycobacterium phlei extract, which has been shown by Brodie et al. (11) to contain a quinone-like compound and to be necessary for oxidative phosphoryla-

| Table I |

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Irradiation period</th>
<th>ΔO₂</th>
<th>ΔP₁</th>
<th>P:O</th>
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</thead>
<tbody>
<tr>
<td>Succinate</td>
<td>min.</td>
<td>16.4</td>
<td>30.2</td>
<td>1.84</td>
</tr>
<tr>
<td>Succinate</td>
<td>30</td>
<td>14.4</td>
<td>24.8</td>
<td>1.72</td>
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<tr>
<td>Succinate</td>
<td>60</td>
<td>6.6</td>
<td>4.2</td>
<td>0.64</td>
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<tr>
<td>Glutamate</td>
<td>0</td>
<td>17.1</td>
<td>44.4</td>
<td>2.50</td>
</tr>
<tr>
<td>Glutamate</td>
<td>30</td>
<td>8.8</td>
<td>8.5</td>
<td>1.04</td>
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<tr>
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<td>60</td>
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<td>3.2</td>
<td>0.77</td>
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| Table II |

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<th>Conditions</th>
<th>Substrate</th>
<th>Additions</th>
<th>ΔO₂</th>
<th>ΔP₁</th>
<th>P:O</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
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<td>16.9</td>
<td>32.2</td>
<td>1.90</td>
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<tr>
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<td>Succinate</td>
<td>K₁ + cytochrome c</td>
<td>16.1</td>
<td>90.6</td>
<td>1.84</td>
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<tr>
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<td>Succinate</td>
<td>None</td>
<td>7.9</td>
<td>5.3</td>
<td>0.67</td>
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<td>Albumin</td>
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<td>Succinate</td>
<td>K₁</td>
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<td>3.9</td>
<td>0.59</td>
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<td>Succinate</td>
<td>Cytochrome c</td>
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<td>23.4</td>
<td>1.59</td>
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<td>Succinate</td>
<td>K₁ + cytochrome c</td>
<td>15.1</td>
<td>22.7</td>
<td>1.50</td>
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<td>Glutamate</td>
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<td>K₁ + cytochrome c</td>
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<td>Glutamate</td>
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<td>Glutamate</td>
<td>Albumin</td>
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<td>2.9</td>
<td>0.71</td>
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<td>Glutamate</td>
<td>DPN</td>
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<td>3.1</td>
<td>0.67</td>
</tr>
<tr>
<td>Irradiated</td>
<td>Glutamate</td>
<td>K₁</td>
<td>9.4</td>
<td>14.6</td>
<td>1.55</td>
</tr>
<tr>
<td>Irradiated</td>
<td>Glutamate</td>
<td>Cytochrome c</td>
<td>4.2</td>
<td>2.8</td>
<td>0.67</td>
</tr>
<tr>
<td>Irradiated</td>
<td>Glutamate</td>
<td>K₁ + cytochrome c</td>
<td>14.9</td>
<td>34.4</td>
<td>2.31</td>
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| Table III |

<table>
<thead>
<tr>
<th>Effect of quinones on oxidative phosphorylation after irradiation</th>
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</thead>
<tbody>
<tr>
<td>All quinones, except Vitamin K₁, Q₂₇₅, and M. phlei extract, were 10⁵ M final concentration. Q₂₇₅ = 0.1 mg per flask; M. phlei extract = approximately 0.05 mg per flask. Substrate, glutamate; all flasks contained cytochrome c, 4 × 10⁻⁶ M.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Additions</th>
<th>ΔO₂</th>
<th>ΔP₁</th>
<th>P:O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.7</td>
<td>38.8</td>
<td>2.64</td>
</tr>
<tr>
<td>Irradiated</td>
<td>5.1</td>
<td>3.1</td>
<td>0.61</td>
</tr>
<tr>
<td>Vitamin K₁</td>
<td>11.6</td>
<td>25.2</td>
<td>2.37</td>
</tr>
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<td>K₁ diacetate</td>
<td>7.4</td>
<td>8.8</td>
<td>0.66</td>
</tr>
<tr>
<td>Menadione</td>
<td>4.3</td>
<td>2.6</td>
<td>0.63</td>
</tr>
<tr>
<td>Menadione diphosphate</td>
<td>3.6</td>
<td>2.4</td>
<td>0.62</td>
</tr>
<tr>
<td>1,4-Naphthaquinone</td>
<td>4.6</td>
<td>2.9</td>
<td>0.63</td>
</tr>
<tr>
<td>Phthioic acid</td>
<td>5.2</td>
<td>3.0</td>
<td>0.58</td>
</tr>
<tr>
<td>p-Quinone</td>
<td>3.3</td>
<td>2.0</td>
<td>0.63</td>
</tr>
<tr>
<td>d,a-Toopherol</td>
<td>4.1</td>
<td>2.2</td>
<td>0.54</td>
</tr>
<tr>
<td>Q₂₇₅</td>
<td>4.9</td>
<td>3.3</td>
<td>0.67</td>
</tr>
<tr>
<td>M. phlei extract</td>
<td>7.9</td>
<td>8.2</td>
<td>1.04</td>
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<tr>
<td>Near-ultraviolet treated vitamin K₁</td>
<td>4.6</td>
<td>2.7</td>
<td>0.59</td>
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</table>
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ultraviolet irradiation, exhibited partial restorative activity.

Other conditions as stated in Experimental section.

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but the same addition to the mitochondrial systems with succinate system partially restored oxidation and phosphorylation, to oxygen. The addition of vitamin K, alone to the glutamate oxidation and accompanying phosphorylations accompanying succinate oxidation. Inhibition of electron transport. The DPN-dependent glutamate oxidation and accompanying phosphorylations were more sensitive to this electron transport. The DPN-dependent transfer of hydrogen from glutamate to cytochrome c. Grabe (33) has provided theoretical evidence from statistical mechanical calculations for such a view, whereby the removal of $2\alpha$-electrons from the benzene ring by the cytochrome complex may bring about activation of the phosphonyl group on position 4. Clark et al. (34) have presented chemical evidence which indicates that such reactions are possible in vitro.

In the present work, near-ultraviolet light has been employed to study the role of light-sensitive compounds in electron transport and accompanying phosphorylations. Near ultraviolet light treatment of mitochondria resulted in a progressive loss of the ability of these particles to oxidize succinate and glutamate as well as to couple efficiently the phosphorylation of ADP to electron transport. The DPN-dependent glutamate oxidation and accompanying phosphorylations were more sensitive to this type of irradiation than the oxidation of succinate and phosphorylations accompanying succinate oxidation. Inhibition of the more resistant oxidative phosphorylation with succinate as substrate was reversed by the addition of cytochrome c to the incubation medium; however, cytochrome c alone did not have any appreciable restorative effect on phosphorylations accompanying the DPN-dependent transfer of hydrogen from glutamate to oxygen. The addition of vitamin K$_1$ alone to the glutamate system partially restored oxidation and phosphorylation, but the same addition to the mitochondrial systems with succinate as substrate was without effect. Both cytochrome c and vitamin K$_1$ were necessary for good restoration of oxidative phosphorylation with glutamate as substrate. These data indicate that a rate-limiting step in electron transport, before the point in electron transfer common to succinoxidase and glutamate oxidase, was inhibited by near-ultraviolet light treatment of mitochondria and that the inhibition before this common point may be reversed by vitamin K$_1$. Inhibition of the cytochrome level was common to both pathways, and thus the presence of cytochrome c was necessary for good restoration of both the succinate and glutamate systems.

A number of quinones other than vitamin K$_1$, as well as an extract of M. phlei exhibiting an absorption spectrum similar to vitamin K$_1$, (11), were tested as to their restorative activities in order to gain information on the specificity of vitamin K$_1$ in this irradiated system. Vitamin K$_1$ diacetate, which differs from vitamin K$_1$ in that the 1 and 4 position carbonyl groups are acetylated, was able to induce slight restoration. This is in agreement with Brodie et al. (11) who found vitamin K$_1$ diacetate to be somewhat restorative in a M. phlei system. A lipidic extract of M. phlei, which, like vitamin K$_1$, restores oxidative phosphorylation from DPNH in M. phlei (11), was also capable of inducing slight restoration in the rat liver mitochondrial system. Q$_{ep}$, a quinone which has been reported to participate in the succinoxidase system (12) was not active in restoring the system after treatment with light in the near-ultraviolet. $d,\alpha$-Tocopherol, which has been reported (13) to be able to restore DPNH-cytochrome c reductase activity after extraction of the enzyme preparation with isooctane, was not active in our system. The absorption spectrum of vitamin K$_1$ was altered by near-ultraviolet light treatment, and after such alteration the vitamin was no longer capable of restoring electron transport with glutamate as substrate. Since it is generally stated that vitamin K$_1$ appears to be limited to green plants and vitamin K$_1$ to microbial systems (35), it does not seem reasonable to claim that vitamin K$_1$ is present in mammalian mitochondria. It is more probable that the vitamin K$_1$ molecule is structurally similar to a component of this system and thus may replace the mitochondrial component after its alteration by near-ultraviolet light.

Cooper and Lehninger (36) suggest that vitamin K$_1$ is not a component of the electron transport system but acts as an intermediate phosphate carrier between electron transport and the final phosphorylation of ADP. Such an interpretation would imply that upon alteration of the intermediate phosphate in a tightly coupled system electron transport would be limited by the lack of available phosphate acceptor. Since electron transport was restored by vitamin K$_1$ in the presence of 2,4-dinitrophenol with glutamate as substrate, the vitamin K$_1$ replaceable factor must function as an electron carrier in electron transport and not solely as an intermediate phosphate carrier. Dicumarol completely inhibits phosphorylation in the restored glutamate systems which is in agreement with Cooper and Lehninger (36) who have reported that Dicumarol blocks phosphorylation in the span $\beta$-hydroxybutyrate to oxygen and $\beta$-hydroxybutyrate to cytochrome c. These latter authors are in disagreement with Martius (5, 15) and Martius and Nitz-Litzow (4) who have offered the hypothesis that Dicumarol acts competitively with vitamin K in oxidative phosphorylation and that vitamin K functions in oxidative phosphorylation solely in the span DPNH to cytochrome c. Our finding that vitamin K$_1$ is able to restore oxidative phosphorylation in the glutamate pathway, but not in the succinate, as well as the finding that the vitamin K$_1$-restored system is sensitive to Amytal, indicates that the step restored by vitamin K$_1$ precedes the point common to electron transport from succinate and DPNH and is thus between DPN and cytochrome b. An alternative hypothesis to both those of Martius (5, 15) and Martius and Nitz-Litzow (4) and Cooper and Lehninger (36) is that quinones function as energy conservation sites in electron transport at all three phosphorylation sites, but that the quinones at each site differ from one another either structurally or in their linkage to adjacent electron carriers. The quinones may be so structurally related as to be inhibited in their phosphorylative roles by Dicumarol, while at the same time only one of the quinones, that replaceable by vitamin K$_1$, is sensitive to alteration in situ by near-ultraviolet light.
SUMMARY

Isolated rat liver mitochondria which have been irradiated with near-ultraviolet light partially lose the ability to oxidize succinate and glutamate and to carry out oxidative phosphorylation. Oxidative phosphorylation with succinate as substrate is restored by near-ultraviolet light treatment of mitochondria alters a factor functioning in oxidative phosphorylation between diphosphopyridine nucleotide and cytochrome b which may be replaced by vitamin K₁.

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REFERENCES

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