by dithionite. The g=1.82 components of Chromatium D (Leigh & Dutton, 1972) and Rps. spheroides (J. S. Leigh & P. L. Dutton, unpublished work) are very temperature-dependent, and the results above suggest that it may be even more so in Rps. capsulata.

Since a direct redox titration of Photoredoxin was not possible, an indirect method, similar to that of Dutton et al. (1973b), was used which involved titrating the magnitude of the light-induced P870⁺ or P870 triplet signal. This estimated the E₉₇ of the primary acceptor of Rps. capsulata to be ~30mV, in good agreement with the estimate of Evans (1973). Since the former titration was carried out at 8°K, whereas the latter was performed at room temperature this would suggest that the low-temperature e.s.r. redox-potentiometric technique is a valid experimental approach for the characterization of components of electron-transport chains.

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A Change in the Type of Interaction of Isatogens with Mitochondrial Energy-Transfer Systems Brought About by Chemical Modification of 2-Phenylisatogen

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Inhibitors have proved useful in the study of mitochondrial oxidative phosphorylation, especially when molecules of well-established structure and chemical properties were used (Beechey & Cattell, 1972). Detailed knowledge of the chemical properties of a compound allows modifications to be made to the molecule, which could result in an increased potency of the inhibitor, or alternatively a change in the pattern of activities observed. The isatogens are a group of compounds that satisfy the above criteria. Sweetman et al. (1971) showed that 2-phenylisatogen inhibited ADP-stimulated succinate oxidation and the 2,4-dinitrophenol-stimulated ATPase (adenosine triphosphatase) activities in rat liver mitochondria. We now report on the effects of a related compound, 2-phenylcarbamoylisatogen, on mitochondrial oxidative phosphorylation.

Table 1 shows a summary of the effects of 2-phenylcarbamoylisatogen and 2-phenylisatogen on some mitochondrial reactions. 2-Phenylisatogen had no effect on respiration in the absence of ADP, but the stimulation of respiration initiated by the phosphate acceptor was completely prevented by low concentrations of the inhibitor. The inhibition of state 3 respiration, caused by 2-phenylisatogen, was restored by the addition of uncoupling concentrations of 2,4-dinitrophenol. A different pattern of activities was found when 2-phenylisatogen was replaced with 2-phenylcarbamoylisatogen. ADP-stimulated respiration was unaffected by 2-phenylcarbamoylisatogen, but there was a marked
Table 1. Effect of 2-phenylisatogen and 2-phenylcarbamoylisatogen on respiration in rat liver mitochondria

Oxygen consumption was measured polarographically with a Clark-type oxygen electrode. The reaction medium contained 675 μmol of sucrose, 10 μmol of Tris-HCl buffer, pH 7.4, 10 μmol of potassium phosphate and rat liver mitochondria (5 mg of protein). Where indicated the following additions were made: ADP (0.5 μmol); oligomycin (2 μg); 2,4-dinitrophenol (30 μmol); 2-phenylisatogen (40 nmol) and 2-phenylcarbamoylisatogen (100 nmol). The temperature was 30°C and the final volume was 3 ml.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Controls</th>
<th>With 2-phenylisatogen</th>
<th>With 2-phenylcarbamoylisatogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>10</td>
<td>10</td>
<td>45</td>
</tr>
<tr>
<td>ADP</td>
<td>54</td>
<td>10</td>
<td>55</td>
</tr>
<tr>
<td>ADP + oligomycin</td>
<td>10</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>57</td>
<td>50</td>
<td>n.t.</td>
</tr>
<tr>
<td>2-Phenylisatogen</td>
<td>10</td>
<td>n.t.</td>
<td>55</td>
</tr>
</tbody>
</table>

stimulation of respiration in the absence of ADP. Whereas 2-phenylisatogen did not alter the rate of oligomycin-inhibited ADP-stimulated respiration, 2-phenylcarbamoylisatogen released the inhibition in a manner similar to that observed with 2,4-dinitrophenol. These findings suggested that the two isatogens were interacting with the mitochondrial energy-transfer system in a different manner. This hypothesis was confirmed by the observation that the inhibition of state 3 respiration caused by 2-phenylisatogen was reversed by the addition of 2-phenylcarbamoylisatogen to the reaction medium.

Measurement of P: O ratios showed that 2-phenylisatogen affected the extent of both oxidation and phosphorylation equally. The stimulation of respiration induced by 2-phenylcarbamoylisatogen was accompanied by an inhibition of ATP synthesis, as measured by a marked decrease in P: O ratios.

Mitochondrial ATPase reactions provide a convenient system for studying the mode of action of energy-transfer inhibitors and uncoupling agents. Rat liver mitochondria catalysed the hydrolysis of ATP at a rate of 0.8 μmol/h per mg of protein. The inclusion of 2-phenylcarbamoylisatogen in the reaction medium resulted in an increase in ATPase activity (the maximal rate was 4.8 μmol/h per mg of protein, in the presence of 150 nmol of isatogen/mg of mitochondrial protein). 2-Phenylisatogen, at concentrations up to 200 nmol/mg of mitochondrial protein, had no effect on the ATPase reaction. The effects of 2-phenylcarbamoylisatogen were similar to those of 2,4-dinitrophenol, whereas 2-phenylisatogen has been shown to inhibit the 2,4-dinitrophenol-stimulated ATPase (Sweetman et al., 1971). The 2-phenylcarbamoylisatogen-stimulated ATPase was inhibited by 2-phenylisatogen and by oligomycin.

The results obtained are consistent with the hypothesis that isatogens exert their effects by an interaction with the mitochondrial energy-transfer system. However, the type of interaction observed is dependent on the structure of the isatogen molecule. The introduction of a carbamoyl group between the isatogen ring and the phenyl ring at the 2-position resulted in a change in activity. The 2-phenyl derivative has been shown to possess energy-transfer inhibitory activity, whereas the 2-phenylcarbamoyl derivative has an uncoupling action. Both effects could arise as a result of combination with the same subunit of the mitochondrial ATPase system.