phosphate, and the possibility that the reaction may also be limited by the stability of other factors, explains the incompleteness of the transcarbamoylation.

In conclusion, these results are consistent with the presence in *S. clavuligerus* of an enzyme which transfers a carbamoyl group from the carbamoyl phosphate to the 3-hydroxymethyl group on the cephalosporin nucleus. This enzyme should be called an O-carbamoyltransferase. We know of no previous description of such an enzyme.

We thank Mr. D. I. Bilkus for his assistance with the high-pressure liquid chromatograms.

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**Effects of (+)-Catechin in vitro and in vivo on Disturbances Produced in Rat Liver Endoplasmic Reticulum by Carbon Tetrachloride**

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The hepatotoxic agent carbon tetrachloride produces major disturbances in the structure and function of liver endoplasmic reticulum very soon after its oral administration to the rat (for reviews dealing with these features of carbon tetrachloride hepatotoxicity, see Recknagel & Glende, 1973; Slater, 1972).

Carbon tetrachloride interacts with the NADPH-cytochrome *P*-450 electron-transfer chain of liver endoplasmic reticulum to produce a highly reactive intermediate, most probably the trichloromethyl radical, CCl₃⁺ (see Slater, 1972, for discussion). By analogy with pulse-radiolysis studies, it is likely that the enzymic process proceeds via electron donation from the NADPH-cytochrome *P*-450 complex to form an adduct, which is followed very rapidly by a dissociative event:

\[
\text{CCl}_4 + e^- \rightarrow \text{CCl}_4^{+} \rightarrow \text{CCl}_3^+ + \text{Cl}^{-} \tag{1}
\]

The trichloromethyl radical is known from previous studies (Willson & Slater, 1975) to have a very high reactivity in solution with biologically important molecules, and is a strongly oxidizing radical. Trichloromethyl radicals, for example, readily withdraw electrons from promethazine (Pr), a well known free-radical scavenger (Willson & Slater, 1975):

\[
\text{CCl}_3^+ + \text{Pr} \rightarrow \text{CCl}_3^- + \text{Pr}^{**} \tag{2}
\]

In a similar manner, the reaction of CCl₃⁺ with a polyunsaturated fatty acid ('PUFAH' in the equations below) in the liver endoplasmic reticulum can be represented thus:

\[
\text{CCl}_3^+ + \text{PUFAH} \rightarrow \text{CCl}_3\text{H} + \text{PUFA}^* \tag{3}
\]

with subsequent reactions that proceed to a peroxidative degradation:

\[
\text{PUFA}^* + \text{O}_2 \rightarrow \text{PUFAO}_2^* \tag{4}
\]

\[
\text{PUFAO}_2^* + \text{PUFAH} \rightarrow \text{PUFAO}_2\text{H} + \text{PUFA}^* \tag{5}
\]

Products of reactions (4) and (5) above → dienes, aldehydes, ethane, etc. \(\tag{6}\)
Table 1. Effects of (+)-catechin on enzyme activities in rat liver microsomal fractions

The results obtained with different concentrations of (+)-catechin are expressed as percentages of the corresponding controls. The results for lipid peroxidation refer to the stimulation in peroxidation produced by carbon tetrachloride as described by Slater & Sawyer (1971a).

<table>
<thead>
<tr>
<th>Concentration of catechin (μM)</th>
<th>NADPH-cytochrome c reductase</th>
<th>Aminopyrene demethylation</th>
<th>Stimulation of lipid peroxidation</th>
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</thead>
<tbody>
<tr>
<td>21</td>
<td>104</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>42</td>
<td>107</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>215</td>
<td>71</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>26</td>
<td>—</td>
<td>113</td>
<td>—</td>
</tr>
<tr>
<td>263</td>
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<td>140</td>
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<td>10</td>
<td>—</td>
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<td>40</td>
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<td>—</td>
</tr>
<tr>
<td>100</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

It is known that carbon tetrachloride stimulates lipid peroxidation in liver endoplasmic reticulum shortly after oral dosing, as measured by diene conjugation (Klaassen & Plaa, 1969; see Recknagel & Glende, 1973) or by malonaldehyde production (Jose & Slater, 1972).

Under conditions in vitro, carbon tetrachloride stimulates lipid peroxidation in liver microsomal fractions, provided that NADPH is present (Slater 1967). The stimulation of lipid peroxidation by carbon tetrachloride is inhibited by a number of free-radical scavengers such as propyl gallate, promethazine and diphenyl-p-phenylene diamine (see Slater, 1972, for references). In an attempt to produce protection against carbon tetrachloride-produced liver injury we have studied the effects of (+)-catechin, a good free-radical scavenger in vitro (Slater & Eakins, 1975) and whose oral LD50 in the rat is approx. 16g/kg.

Adult male albino rats (body wt. 180-200g) were obtained from R. Tuck and Sons, Rayleigh, Essex, U.K. They were anaesthetized with ether, and blood was taken from the abdominal aorta; rats were killed by cervical dislocation, and a microsomal fraction was isolated as described previously (Slater & Sawyer, 1971a). (+)-Catechin was given intraperitoneally as a fine suspension in water; the pH was adjusted to 7.4 with alkali and the dose administered was 100mg/kg body wt. Sodium ascorbate (pH 7.4) was given intraperitoneally at a dose of 500mg/kg body wt. Carbon tetrachloride was administered by stomach tube (1.25ml/kg body wt. in liquid paraffin, 1:3, v/v) 15min after dosing with either or both (+)-catechin and ascorbate. Cytochromes P-450 and b5 were measured as described by Slater & Sawyer (1969), liver triglyceride by the method of Van Handel et al. (1957) modified by Ugazio et al. (1971), microsomal protein by the method of Lowry et al. (1951), with bovine plasma albumin as standard; the following enzyme activities in serum were measured by using test-kits obtained from Boehringer U.K. Ltd.: sorbitol dehydrogenase, lactate dehydrogenase and isocitrate dehydrogenase. The effects of (+)-catechin on microsomal aminopyrene demethylation, NADPH-cytochrome c reductase and on the stimulatory effect of carbon tetrachloride on microsomal lipid peroxidation were measured by using the procedures of Slater & Sawyer (1971b), Slater & Sawyer (1969) and Slater & Sawyer (1971a) respectively. The results were evaluated by using Student's t test, making allowance where relevant for the non-normal distribution of some serum enzyme activities (Heath, 1967).

Under conditions in vitro, (+)-catechin had no inhibitory effect on NADPH-cytochrome c reductase except at rather high concentration (200μM); it produced a small increase in the rate of aminopyrene demethylation and a strong inhibition of the stimulation of lipid peroxidation by carbon tetrachloride (see Table 1).
Table 2. Effects of vitamin C (Vit. C) and (+)-catechin on parameters of liver damage produced by carbon tetrachloride in vivo

The units used are as follows: protein (Prot.), mg/liver per 100 g body wt.; cytochromes P-450 (P-450) and b₅ (Cyt.b₅), nmol/liver per 100 g body wt.; sorbitol dehydrogenase (SDH), munits/ml; isocitrate dehydrogenase (ICDH), units/ml; lactate dehydrogenase (LDH), units/ml; triglyceride (TG), mg/liver per 100 g body wt. Statistical evaluation is shown for group (b) versus group (a) and for groups (c and d) versus group (b). LP, Liquid paraffin; N.D., not determinable; *P<0.05; †P<0.025; ‡P<0.001.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>3h</th>
<th>6h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prot</td>
<td>P-450</td>
<td>Cyt.b₅</td>
<td>SDH</td>
</tr>
<tr>
<td>(a) NaCl+LP</td>
<td>85.97</td>
<td>1.86</td>
<td>2.70</td>
<td>5.07</td>
</tr>
<tr>
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<td>± 3.81</td>
<td>0.15</td>
<td>0.36</td>
<td>0.88</td>
</tr>
<tr>
<td>(b) NaCl+CCI₄</td>
<td>91.87</td>
<td>†1.19</td>
<td>2.63</td>
<td>†12.68</td>
</tr>
<tr>
<td></td>
<td>± 3.54</td>
<td>0.18</td>
<td>0.47</td>
<td>0.88</td>
</tr>
<tr>
<td>(c) Vit.C+CCI₄</td>
<td>91.60</td>
<td>0.86</td>
<td>2.51</td>
<td>†21.86*22.43</td>
</tr>
<tr>
<td></td>
<td>± 3.57</td>
<td>0.13</td>
<td>0.29</td>
<td>2.66</td>
</tr>
<tr>
<td>(d) Vit.C+catechin +CCI₄</td>
<td>87.10</td>
<td>1.05</td>
<td>2.47</td>
<td>15.59</td>
</tr>
<tr>
<td></td>
<td>± 3.98</td>
<td>0.12</td>
<td>0.41</td>
<td>2.58</td>
</tr>
</tbody>
</table>
Under conditions in vivo (Table 2), catechin or ascorbate, alone or in combination, had no protective action on a number of parameters of liver injury over the period 3–24 h after the administration of carbon tetrachloride. In other experiments (not quoted here in full) we have found that (+)-catechin does not prevent the decrease in the amount of whole-liver NADPH normally seen 1 h after carbon tetrachloride administration (Slater et al., 1964). The results obtained are unusual in that although (+)-catechin is a powerful scavenger of free radicals in vitro (Slater & Eakins, 1975) and inhibits the stimulation of lipid peroxidation by carbon tetrachloride in vitro (Table 1), it does not exert protective action in vivo as assessed by the parameters used in the present study. The effects of (+)-catechin in vivo were not improved by the co-administration of ascorbate, which was used to inhibit oxidative degradation of catechin in solution.

(+)-Catechin is metabolized by the liver (Das & Griffiths, 1968, 1969) and is rapidly excreted in the bile (Das & Sothy, 1971). The biliary concentration is maximal about 1 h after intravenous injection and rapidly falls over the subsequent 60 min. It is therefore likely that appreciable liver concentrations of (+)-catechin were obtained for only 1–2 h after dosing, and could thus be expected at the most only a moderate delay on the carbon tetrachloride-mediated liver injury. The results obtained, however, give no indication of any protective delaying effect for (+)-catechin in vivo.

The results obtained here are somewhat similar to those previously found for vitamin E, which is also effective in vitro and yet virtually inactive in vivo against carbon tetrachloride-mediated liver injury (for references, see Slater, 1972; Ghoshal, 1976). The failure of vitamin E to exert protective effects in vivo has been attributed to its inability to penetrate to the precise locus of CCl₄ formation in the endoplasmic reticulum (Slater, 1976); calculations indicate that CCl₄ has a very small diffusion radius in the membranes of the endoplasmic reticulum (Slater, 1976). It is possible that the lack of effect of (+)-catechin in vivo in the system studied here is due to a combination of its short residence time in the liver, together with a failure to penetrate to the precise intra-cellular locus of carbon tetrachloride activation.

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