REVERSAL OF RESPIRATORY DECLINE IN NECROTIC LIVER DEGENERATION BY INTRAPORTAL TOCOPHEROLS

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Necrotic liver degeneration is produced in rats by diets deficient in vita-
min E, cystine, and Factor 3 (1). During the latent phase of the disease, several weeks before the onset of acute hepatic necrosis, a defect in oxidative metabolism has been demonstrated in livers of animals on such diets. Oxygen consumption of histologically unaffected liver slices breaks down after a brief initial period of normal respiration (2). This decline of O₂ uptake is associated with an impaired utilization of acetate-C¹⁴ for lipogenesis, ketogenesis, and CO₂ formation (3). The agents preventing hepatic necrosis, vitamin E, cystine, or Factor 3, also forestall the respiratory lesion effectively when supplemented to the diet.

The addition in vitro of α-tocopherol to liver slices from deficient rats, either as an emulsion or in water-soluble form, has no significant effect on the metabolic lesion. However, preliminary studies showed that injection of emulsions of dl-α-tocopherol into the portal vein caused the almost immediate disappearance of the defect (4). The present report describes this phenomenon; the relative potencies of several forms of α-tocopherol and of the other tocopherols (β-, γ-, and δ-) are compared. Both α- and γ-tocopherol were found to be quite potent in their ability to reverse the metabolic lesion upon intraportal injection; β- and δ-tocopherol were much less so.

EXPERIMENTAL

Male, weanling Sprague-Dawley rats of the National Institutes of Health strain, 18 to 22 days old, were fed a basal vitamin E-free diet² containing Torula yeast as the sole source of protein for periods of 24 to 59 days (body weight 60 to 120 gm.). The average survival time of rats of this strain

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1 Unpublished experiments.

2 The basal diet contained 30 per cent Torula yeast, 59 per cent sucrose, 5 per cent vitamin E-free lard, 5 per cent salts, and 1 per cent vitamin mixture (2, 5).
fed this diet is about 45 days under the experimental conditions employed here (5).

Anesthesia was induced by intravenous or intraperitoneal injection of sodium pentobarbital (2.5 mg. per 100 gm. of body weight). The abdomen of the animal was opened, and a small lobe of liver removed for the preparation of preinjection control slices. 0.2 to 0.5 ml. of emulsion at body temperature was injected directly into the portal vein and the abdomen closed. After 30 minutes the animal was sacrificed by decapitation, the liver examined for evidence of necrotic degeneration, and postinjection slices prepared. Each animal thus served as its own control.

The areas taken for slices were free of necrosis (2). Slices were prepared free-hand, blotted free of excess moisture, and weighed. Duplicate samples of approximately 100 mg. were incubated in Warburg vessels containing 3 ml. of oxygenated Krebs-Ringer-phosphate buffer (pH 7.4) with 0.01 m glucose (6). Bath temperature was maintained at 37.5° and readings were made at 10 to 20 minute intervals for 2 to 3 hours. Oxygen consumption was calculated in terms of microliters of oxygen consumed per 100 mg. of fresh weight of slices per hour of incubation (QO2 (F 100)).

Preparation of Tocopherol Solutions—A water-soluble preparation of \( d-\alpha \)-tocopherol, designated as \( d-\alpha \)-tocopheryl polyethylene glycol-1000 succinate, containing approximately 30 per cent, by weight, of \( \alpha \)-tocopherol, was made up in 0.9 per cent NaCl. The free \( \alpha \)-tocopherol employed was either the synthetic, racemic material,\(^3\) or \( d-\alpha \)-tocopherol of natural origin. The other tocopherols were the free, natural alcohols.\(^4\) The highly viscid oils were diluted with olive oil, which contains negligible amounts of vitamin E (7). 0.5 ml. of olive oil, 100 mg. of glycerol monostearate, and 9.5 ml. of 5 per cent glucose were warmed and homogenized in a tube with a tight fitting Teflon pestle. The mixtures were homogenized before each injection.

**Results**

The intraportal administration of emulsions of olive oil, glycerol monostearate, and glucose, but without tocopherol, did not affect the respiratory decline (Table I). Addition to the emulsion of small amounts of \( \alpha \)- or \( \gamma \)-tocopherol, or of larger amounts of \( \beta \)- or \( \delta \)-tocopherol, resulted in sustained maintenance of oxygen uptake by the liver slices for 2 hours or longer. The presence of necrotic areas in the liver did not preclude the response to injected tocopherol by the slices, prepared from grossly and histologically

\(^3\) The racemic \( \alpha \)-tocopherol was purchased from Merck and Company, Rahway, New Jersey.

\(^4\) The naturally occurring tocopherols were purchased from Distillation Products, Inc., Rochester, New York. We are indebted to Dr. P. L. Harris for the \( d-\alpha \)-tocopheryl polyethylene glycol-1000 succinate.
normal parts of the organ. The rate of initial respiration of the postinjection slices was, in general, not significantly greater than that of the preinjection control preparations. When an apparent increase in the initial 30 minute respiration of postinjection slices was noted, it was usually found that the oxygen consumption of the preinjection control slices had declined

### TABLE I

**Effect of Intraportal α-Tocopherol on Respiratory Decline of Liver Slices**

<table>
<thead>
<tr>
<th>α-Tocopherol injected, mg.</th>
<th>No. of rats</th>
<th>No. of rats with hepatic necrosis</th>
<th>( \text{QO}_2 (\text{F W't/mg}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Preinjection control</td>
</tr>
<tr>
<td>Olive oil emulsion†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6</td>
<td>4</td>
<td>251 ± 46</td>
</tr>
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</table>

#### dl-α-Tocopherol emulsion

<table>
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<th></th>
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<th>(4)</th>
<th>(5)</th>
<th>(6)</th>
<th>(7)</th>
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<tbody>
<tr>
<td>(&lt;0.05 )</td>
<td>8</td>
<td>7</td>
<td>224 ± 24</td>
<td>83 ± 32</td>
<td>239 ± 38</td>
<td>119 ± 55</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>12</td>
<td>7</td>
<td>225 ± 40</td>
<td>62 ± 48</td>
<td>248 ± 40</td>
<td>151 ± 63$</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>25</td>
<td>17</td>
<td>217 ± 38</td>
<td>66 ± 27</td>
<td>226 ± 51</td>
<td>145 ± 54$</td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>3</td>
<td>2</td>
<td>261 ± 19</td>
<td>105 ± 29</td>
<td>310 ± 49</td>
<td>261 ± 29$</td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>4</td>
<td>3</td>
<td>254 ± 40</td>
<td>94 ± 52</td>
<td>284 ± 20</td>
<td>258 ± 37$</td>
<td></td>
</tr>
<tr>
<td>( &gt;1.0 )</td>
<td>4</td>
<td>3</td>
<td>205 ± 11</td>
<td>70 ± 30</td>
<td>246 ± 61</td>
<td>226 ± 69$</td>
<td></td>
</tr>
</tbody>
</table>

#### d-α-Tocopherol emulsion

<table>
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<th></th>
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<th>(4)</th>
<th>(5)</th>
<th>(6)</th>
<th>(7)</th>
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</thead>
<tbody>
<tr>
<td>0.1</td>
<td>5</td>
<td>1</td>
<td>236 ± 30</td>
<td>110 ± 55</td>
<td>285 ± 25</td>
<td>180 ± 35</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>10</td>
<td>2</td>
<td>244 ± 64</td>
<td>111 ± 55</td>
<td>290 ± 45</td>
<td>226 ± 63$</td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>4</td>
<td>0</td>
<td>242 ± 59</td>
<td>68 ± 22</td>
<td>325 ± 49</td>
<td>267 ± 51$</td>
<td></td>
</tr>
</tbody>
</table>

#### d-α-Tocopherol polyethylene glycol-1000 succinate

<table>
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<th>(4)</th>
<th>(5)</th>
<th>(6)</th>
<th>(7)</th>
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<tbody>
<tr>
<td>0.05</td>
<td>3</td>
<td>2</td>
<td>269 ± 56</td>
<td>36 ± 20</td>
<td>251 ± 39</td>
<td>113 ± 28$</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>8</td>
<td>3</td>
<td>232 ± 52</td>
<td>129 ± 67</td>
<td>252 ± 31</td>
<td>218 ± 38$</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>8</td>
<td>3</td>
<td>223 ± 38</td>
<td>100 ± 48</td>
<td>264 ± 39</td>
<td>213 ± 51$</td>
<td></td>
</tr>
</tbody>
</table>

* \( \text{O}_2 \) (c.mm.) consumed per hour per 100 mg. of fresh weight of slices, calculated for first and fourth 30 minute incubation periods.
† Mean and standard deviation.
‡ The emulsions contained 9.5 ml. of 5 per cent glucose, 100 mg. of glycerol monostearate, and 0.5 ml. of olive oil; tocopherols were dissolved in olive oil. 0.2 to 0.5 ml. of emulsions injected intraportally.
§ The differences between the values in Columns 5 and 7 are significant; \( p <0.02 \).
appreciably even within this brief period. There was no evidence of a
direct stimulation of respiration by the tocopherols.

dl-\(\alpha\)-Tocopherol—In Table I the oxygen consumption of liver slices from
rats on the basal diet is compared with that of slices from these same ani-
imals 30 minutes after the intraportal injection of \(dl-\alpha\)-tocopherol emul-
sions. 56 rats received such emulsions; thirty-nine of the rats were found
to have areas of hepatic necrosis. The liver appeared to be normal in the
others. About 0.1 mg. of \(dl-\alpha\)-tocopherol evidently altered the O\(_2\) uptake
of postinjection slices. The decline in respiration during the 2nd hour was
partially prevented, as indicated by the differences between the values for
the pre- and postinjection 90 to 120 minute intervals. However, doses of
more than 0.4 mg. were necessary to maintain for 2 hours the respiration
of postinjection slices at about their initial (30 minute) rate. Above this
level, no significant differences existed between the postinjection values for
0 to 30 minutes, and 90 to 120 minutes of incubation.

d-\(\alpha\)-Tocopherol—The data obtained with d-\(\alpha\)-tocopherol prepared as an
emulsion for intraportal injection compare well with those reported for the
racemic mixture. A highly significant difference in the respiration of pre-
and postinjection slices was observed 90 to 120 minutes after injection of
0.2 mg. of the \(d-\alpha\)-tocopherol emulsion (\(p < 0.01\)). Whether the \(d\) form
is more active than the \(dl-\alpha\)-tocopherol cannot be concluded from these
data.

Water-Soluble \(d-\alpha\)-Tocopheryl Polyethylene Glycol-1000 Succinate—This
substance was administered to nineteen animals in doses of from 0.05 to
0.2 mg. Eight of these rats displayed gross evidence of hepatic necrosis,
while the liver appeared normal in the others. The results are summarized
in Table I. As little as 0.05 mg. of \(\alpha\)-tocopherol in this form significantly
affected the respiratory decline of slices from deficient rats, and 0.1 mg.
maintained the oxygen uptake at approximately the initial 30 minute rate
for the 2 hour incubation period.\(^5\)

d-\(\beta\)-Tocopherol—Fifteen rats were injected with 0.2 to 1.0 mg. of \(d-\beta\)-
tocopherol in an emulsion (Table II). Twelve of the animals had gross
liver damage. Administration via portal vein of less than 0.5 mg. did not
affect the respiratory decline of liver slices. As much as 1.0 mg., while
maintaining the oxygen consumption for somewhat longer periods, did not
prevent marked decline in the rate of O\(_2\) consumption; in six of the nine
instances, the value during the 90 to 120 minute period was less than 80
per cent of the initial 30 minute \(Q_{O_2}\) (F 100).

d-\(\gamma\)-Tocopherol—In Table II are recorded the results obtained with thirty

\(^5\) When added directly to the Warburg medium, as much as 0.7 mg. of \(\alpha\)-tocopherol
in the water-soluble form did not prevent the decline in respiration of liver slices from
deficient rats.
rats injected intraportally with 0.1 to 3.2 mg. of d-γ-tocopherol in an emulsion. Eighteen of the rats showed gross necrotic degeneration at the time of the experiment. 0.2 mg. protected against respiratory decline of the liver slices and 0.8 mg. maintained respiration of postinjection liver slices approximately at their initial respiratory level for the full 2 hour incubation.

**Table II**

*Effect of Intraportal β-, γ-, and δ-Tocopherols on Respiratory Decline of Liver Slices*

<table>
<thead>
<tr>
<th>Tocopherol Injected, mg.</th>
<th>No. of rats</th>
<th>No. of rats with hepatic necrosis</th>
<th>QO₂ (F 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
</tr>
<tr>
<td>β-Tocopherol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>2</td>
<td>2</td>
<td>281 ± 35</td>
</tr>
<tr>
<td>0.5</td>
<td>4</td>
<td>3</td>
<td>269 ± 18</td>
</tr>
<tr>
<td>1.0</td>
<td>9</td>
<td>7</td>
<td>252 ± 30</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>6</td>
<td>5</td>
<td>240 ± 65</td>
</tr>
<tr>
<td>0.2</td>
<td>6</td>
<td>3</td>
<td>238 ± 29</td>
</tr>
<tr>
<td>0.4</td>
<td>6</td>
<td>2</td>
<td>238 ± 28</td>
</tr>
<tr>
<td>0.8</td>
<td>4</td>
<td>4</td>
<td>288 ± 57</td>
</tr>
<tr>
<td>1.6</td>
<td>4</td>
<td>1</td>
<td>290 ± 55</td>
</tr>
<tr>
<td>3.2</td>
<td>4</td>
<td>3</td>
<td>240 ± 56</td>
</tr>
<tr>
<td>δ-Tocopherol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2-1.0</td>
<td>9</td>
<td>3</td>
<td>253 ± 43</td>
</tr>
<tr>
<td>2.0</td>
<td>8</td>
<td>5</td>
<td>274 ± 25</td>
</tr>
</tbody>
</table>

* For the experimental conditions, see Table I.
† The difference between values in Columns 5 and 7 is significant; p < 0.02.

**d-δ-Tocopherol**—The seventeen rats injected intraportally with an emulsion of δ-tocopherol received doses of 0.2 to 2.0 mg. Eight of the animals had gross liver necrosis at the time of sacrifice. Injection of 1.0 mg. or less had virtually no effect on the decline of respiration, as compared with values for preinjection control slices. 2.0 mg., however, prevented the respiratory decline during the 2 hour incubation period.

**Comparative Activities of Tocopherols**—An evaluation of the dose-response curves derived from the data presented in Tables I and II is presented in Table III. Least square lines were fitted, connecting the per cent rever-
sion and the logarithm of the dose of tocopherol. The doses producing a 50 per cent reversion of the respiratory lesion were estimated from the computed constants of these lines. The relative activities of the various preparations tested indicate that the \(d\alpha\)-tocopheryl polyethylene glycol-1000 succinate is more than twice as active as \(d\alpha\)- or \(dl\alpha\)-tocopherol in reverting the respiratory decline of the preinjected control slices. The \(d\alpha\)-tocopherol did not differ from racemic \(\alpha\)-tocopherol. When the activity of \(dl\alpha\)-tocopherol is taken as 100, the relative activities of the other tocopherols were as follows: \(d\gamma\), 83; \(d\beta\), 36; and \(d\delta\), 17.

**Table III**

*Relative Activities of Various Tocopherols for 50 Per Cent Reversion of Respiratory Decline*

<table>
<thead>
<tr>
<th>Tocopherol tested</th>
<th>Dose producing 50 per cent reversion of respiratory lesion</th>
<th>Relative activity ((dl\alpha)-tocopherol = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ( \pm ) 68 per cent confidence limits</td>
<td></td>
</tr>
<tr>
<td>(d\alpha)-Tocopheryl polyethylene glycol-1000 succinate</td>
<td>0.067, 0.049-0.092</td>
<td>277</td>
</tr>
<tr>
<td>(dl\alpha)-Tocopherol</td>
<td>0.190, 0.186-0.195</td>
<td>100</td>
</tr>
<tr>
<td>(d\alpha)-Tocopherol</td>
<td>0.174, 0.148-0.205</td>
<td>109</td>
</tr>
<tr>
<td>(d\gamma)-Tocopherol</td>
<td>0.230, 0.154-0.344</td>
<td>88</td>
</tr>
<tr>
<td>(d\beta)-Tocopherol</td>
<td>0.533, 0.419-0.679</td>
<td>36</td>
</tr>
<tr>
<td>(d\delta)-Tocopherol</td>
<td>1.100, 0.920-1.284</td>
<td>17</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The development of dietary necrotic liver degeneration in the rat is prevented by the feeding of physiological amounts of tocopherol (8). A daily intake of about 0.05 mg. of synthetic \(\alpha\)-tocopheryl acetate is required for 50 per cent protection under our experimental conditions (1). Selzer et al. (9) noted that the weekly oral administration of 0.4 mg. of \(dl\alpha\)-tocopheryl acetate, but not 0.8 mg. of \(\gamma\)- or \(\delta\)-tocopherol, prevented hepatic necrosis in 40 to 90 per cent of rats maintained on a 10 per cent food yeast, 90 per cent corn-starch diet. When tested by the resorption-sterility method in the rat, the relative biopotencies of orally administered, natural \(\alpha\), \(\beta\), \(\gamma\), and \(\delta\)-tocopherols are approximately 100:40:8:1 (10). Only slightly different ratios are obtained by other bioassay methods (11–14).

Knowledge of the absorption and tissue distribution of various tocopherols is far from complete, but the available evidence indicates that the greater biopotency of oral \(\alpha\)-tocopherol is related to a better absorption from the gastrointestinal tract (15–17). In the present study, absorption...
has been bypassed by the injection of emulsions of the free tocopherols into the portal vein. It is clearly seen that as little as 0.1 to 0.2 mg. of d- or dl-α-tocopherol serves to reverse the defective metabolic situation demonstrable in liver slices immediately before injection. The d-α-tocopheryl polyethylene glycol-1000 succinate, when injected intraportally as a clear suspension in water, was found to be more than twice as effective as dl-α-tocopherol. The emulsion of the free tocopherol was much less dispersed, which may explain this difference in activity. Harris and Ludwig reported that both natural and synthetic esters of α-tocopherol, when given orally, are superior in potency to the respective free tocopherols (18).

When emulsions of different tocopherols were compared in our system, d-γ-tocopherol was almost as potent as α-tocopherol in the reversal of the metabolic lesion. This contrasts clearly with the observations made upon oral administration in other vitamin E assays (10). It tends to support the proposition that absorption from the intestine limits the oral biopotency of γ-tocopherol (17). β- and δ-tocopherol, on the other hand, were as ineffective in the intraportal test as they were orally.

Since the site and the mode of action of vitamin E in metabolism are yet unknown, it is not possible to discuss the difference between various tocopherols in terms of biochemical specificity. In our experiments several physicochemical factors other than chemical specificity could contribute to the observed dissimilarities. A vitamin E molecule in trans situ (19) may be subject to oxidation and other chemical influences. It is bound to blood proteins (20) and thus may be carried off by the blood stream, whence it can be taken up by body fat or by other tissues of the vitamin E-deficient animal. Cellular permeability may also be involved. There are indications that, in our system, the action of tocopherol may take place in the mitochondria (2); the substance may have to reach this location before any effects become evident.

**SUMMARY**

1. The effects of intraportal injections of various tocopherols on the respiratory defect of liver slices in dietary necrotic liver degeneration were investigated. Emulsions of various tocopherols and water-soluble d-α-tocopheryl polyethylene glycol-1000 succinate did not markedly alter the initial rate of oxygen consumption, but reversed the respiratory decline, as evident from the pre- and postinjection oxygen consumption after 90 to 120 minutes of incubation. The degree of decline of oxygen consumption diminished with increasing doses of tocopherol.

2. A dose of 0.19 mg. of emulsified dl-α-tocopherol was required for 50 per cent reversion of the respiratory lesion. The d-α-tocopheryl polyethylene glycol-1000 succinate was more than twice as active. d-γ-Tocopherol,
upon intraportal injection, was almost as active as d- or dl-α-tocopherol in reversing the respiratory defect. For 50 per cent reversion, the relative activities were d-α-, 109; d-γ-, 83; d-β-, 36; and d-δ-, 17; these are compared to dl-α-tocopherol as 100.

BIBLIOGRAPHY

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