A Possible Site of Action for Vitamin E in Intermediary Metabolism

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As a corollary to Dr. Caputto's presentation, I would like to discuss briefly the crucial points of some work carried out in the Section on Experimental Liver Diseases with the aim to identify the active site of tocopherol in enzyme systems. This work has been in progress since 1952 in conjunction with our systematic attempts to elucidate the causal chain of events leading from dietary deficiency and metabolic defects on the molecular level to liver necrosis and death. It was discovered early that liver slices from rats on vitamin E deficient diets are incapable of maintaining normal oxygen consumption in the Warburg respirometer for more than thirty to sixty minutes (Fig. 1). The phenomenon, respiratory decline, is characteristic of the latent phase of the disease which precedes liver necrosis by ten to fourteen days. During this phase gross or even microscopic changes are not detectable but serious damage to mitochondria and microsomes is evident from electron microscope pictures. Respiratory decline is indicative of a specific function of tocopherol in the maintenance of normal energy metabolism. The impairment is prevented by feeding vitamin E; it disappears within ten minutes after injection of physiologic amounts of α-tocopherol into the portal vein or into peripheral vessels.

It would not be feasible to discuss these studies here in detail. Some of the results are mentioned elsewhere in this monograph. From the Section on Experimental Liver Diseases, LNE, NIAMD National Institutes of Health, Bethesda, Maryland.

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A thorough analysis of the effects of thirteen different antioxidants has shown that a few of them are quite potent as dietary supplements, and also following intraportal application, while the majority of “run of the mill” antioxidants, especially those which are used commercially for the stabilization of fats, are inactive. When added to the slice medium directly in form of emulsions, tocopherol is completely inactive, while those antioxidants which are effective after injection are also effective in vitro. The observations indicated to us several years ago (1956) that tocopherol is converted in intermediary metabolism into an active form.

We have searched for this active form by testing of various tocopherol derivatives. Thus far, the only substance derived from tocopherol and effective in the prevention of respiratory failure in our in vitro experiments with liver slices is the metabolite described by Simon et al. The material prevents respiratory decline when added at relatively low amounts. Fifty per cent prevention will occur with roughly 6 μg. of the Simon metabolite per 3 cc. of medium and 100 mg. of slices (Table 1). Parenthetically, with 100 μg. or more of the metabolite, a pronounced stimulation of respiratory activity has been observed. From a comparison of the bio-potency of various substances effective in our system, we feel that the Simon metabolite, which is excreted in the urine as the glucuronide, is not the active form of vitamin E. But it seems to be related to it, possibly as a breakdown product of the active compound.

Approximately one and a half years ago it was detected by our group* that phosphate-buffered media permit the observation of respiratory decline in homogenates of vitamin...
α-tocopherol to the medium was without effect, similar to the results obtained by addition of tocopherol directly to liver slices. However, when the vitamin was rehomogenized with the liver homogenate, protection was achieved. The tocopherol metabolite of Simon et al., and also menadione, methylene blue, and especially DPPD were found to be potent agents in the prevention of the metabolic lesion.

Preceding studies had shown that deficient mitochondria alone were metabolically intact and did not respond to conditions of the in vitro experiment with loss of respiratory activity, in contrast to the whole homogenate. The only defect detectable in the mitochondria of E deficient animals was seen in succinate utilization in the presence of DPN. In this case respiration started out relatively fast but came to a standstill in a short period of time. The phenomenon was shown to be correlated to an increased accumulation of oxalacetic acid. The latter is a potent negative feedback inhibitor of succinic dehydrogenase. The exact reason for the enhanced accumulation, or rather the apparent decreased removal of oxalacetic acid from the system has not been clarified.

The results with mitochondria alone, when compared to those with liver homogenates or liver slices, showed clearly that an interaction between various components of the liver cell was involved in the elicitation of respiratory failure. By combining various cell fractions after differential centrifugation it was shown that the microsomal portion greatly accelerated the respiratory failure when combined with the mitochondria while the supernatant had little or no effect. The mechanism of this interaction is not entirely understood at present. Thus far, attempts to extract inhibitory agents from microsomal preparations have led only to partial success. As in Dr. Caputto's system, a trace element seems to be involved.

![Graph](https://academic.oup.com/ajcn/article-abstract/9/4/71/4829500)

**Fig. 1** Respiratory decline of liver slices from rats during the latent phase of dietary necrotic liver degeneration.

E deficient livers. This system has been used for a new approach to the problem at hand. It permits analysis of variables which could not be tested in liver slices. Variation of substrates, especially of members of the Krebs cycle, showed that respiratory decline is a general phenomenon; it occurs also with glutamate, β hydroxybutyrate, etc. With certain substrates tocopherol dependent decline is more rapid and more pronounced than with others. For instance, pyruvate-malate and α-ketoglutarate presented a large degree of imbalance while citrate and succinate were affected least. Supplementation of unchanged

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**Fig. 2.** Urinary tocopherol metabolite isolated by Simon et al.
In Vivo Effect of Tocopherol Derivatives on Respiratory Decline (Liver Slices)\(^*\)

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Dose in Flask</th>
<th>No. of Experiments</th>
<th>Respiratory Decline (%)</th>
<th>Prevention (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tocopherol-metabolite (free acid, quinone form)</td>
<td>6.25</td>
<td>5</td>
<td>73</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>25.00</td>
<td>4</td>
<td>71</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>100.00</td>
<td>5</td>
<td>70</td>
<td>4</td>
</tr>
<tr>
<td>DL-(\alpha)-Tocopherol</td>
<td>200.00</td>
<td>5</td>
<td>72</td>
<td>66</td>
</tr>
<tr>
<td>d-(\alpha)-Tocopheryl polyethylene glycol-1000 succinate</td>
<td>1000.00</td>
<td>5</td>
<td>75</td>
<td>72</td>
</tr>
<tr>
<td>DL-(\alpha)-Tocopherylhydroquinone</td>
<td>200.00</td>
<td>5</td>
<td>71</td>
<td>77</td>
</tr>
<tr>
<td>DL-(\alpha)-Tocopherolquinone</td>
<td>200.00</td>
<td>4</td>
<td>72</td>
<td>74</td>
</tr>
<tr>
<td>DL-(\alpha)-&quot;Tocopheroxide&quot; (acetal of quinone)</td>
<td>200.00</td>
<td>4</td>
<td>67</td>
<td>77</td>
</tr>
</tbody>
</table>

Prevention of Decline of a-Ketoglutarate Oxidation by GSH and BAL (Liver Homogenate)\(^*\)

<table>
<thead>
<tr>
<th>Addition ((\mu)M)</th>
<th>No. of Rats</th>
<th>Time Interval (min.; (\mu)atoms 0/50 mg.)</th>
<th>Decline (%)(^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 GSH</td>
<td>9</td>
<td>0-30' 10.5 ± 0.5 3.2 ± 0.4 69 ± 3</td>
<td>65 ± 3</td>
</tr>
<tr>
<td>0.3 BAL</td>
<td>9</td>
<td>0-30' 8.5 ± 0.3 9.1 ± 0.3 65 ± 12</td>
<td>65 ± 12</td>
</tr>
<tr>
<td>0.3 BAL</td>
<td>5</td>
<td>0-30' 8.5 ± 0.3 9.1 ± 0.3 65 ± 12</td>
<td>65 ± 12</td>
</tr>
</tbody>
</table>

\(*\) Experiment by L. M. Corwin. \(\dagger\) Decline (\%) = 100 \times (0-30') - (60-90')/0-30'. \(\dagger\) = per cent increase.

Following the discovery by McLean that EDTA prevents respiratory decline in liver slices,\(^{11}\) it was shown that EDTA and other complexing agents prevented the respiratory breakdown in our homogenate system as well.\(^*\)

The specific nature of the element which might be involved has not been established conclusively, but it seems likely that iron is the effective ingredient.

It is interesting to note that the metabolic impairment in liver slices is also prevented by reduced glutathione and by BAL. Relatively small amounts of these agents are likewise effective in our homogenate system. BAL is more active than the glutathione-SH (Table\(^{11}\)). In separate studies it was shown by inhibition analysis that the chain of electron carriers does not seem to be involved directly in respiratory decline. It was concluded that the systems which are primarily affected, most likely through attack and inactivation by a trace element, are those dealing immediately with the substrates; i.e., the various dehydrogenase systems. These are all sulfhydryl enzymes.

The various pieces of evidence conveyed here can be integrated to indicate that tocopherol may have a physiologic function in close relationship to thiol or dithiol groups of enzyme systems. The exact nature of this interaction on the molecular level remains to be clarified. From the fact that the vitamin itself is inactive, while the Simon metabolite is effective, one could conclude that not \(\alpha\)-tocopherol but rather a conversion product of the latter participates in the metabolic reaction. It is noteworthy that the glutathione content of liver tissue in animals maintained on our basal diet is greatly reduced compared...
tocols. The dehydrogenase itself contains vicinal dithiol groups in the form of thiotic acid at its active site. The \(\alpha\)-ketoglutarate oxidase system also contains various other sulphhydryl groups. It is particularly sensitive to poisoning by reagents such as arsenite or cadmium. The mechanism of this inhibition and its prevention by BAL and other dithiol compounds has been analyzed by Sanadi and others. They correlated the inhibition to the fact that the dehydrogenase contains the vicinal dithiol groups of thiotic acid. Inhibition is caused by reaction of the metals with the dithiol groups. In our laboratory it has been established by Corwin that the inhibitory effect of arsenite and cadmium on \(\alpha\)-ketoglutarate oxidation of normal liver homogenates can readily be antagonized by those agents which are effective in the prevention of respiratory decline, specifically by rehomogenized tocopherol (Table IV).

The protective effect of the metabolically active form of tocopherol and of the other agents effective in our system could be interpreted in the following two ways:

1. The active compounds, in the oxidized form, i.e., quinones, could interact with sulphhydryl groups at the active sites by an oxidation-reduction reaction which forces the equilibrium towards the S-S form. Such a shift in the equilibrium would effectively eliminate the point of attack of the small amounts of inhibitory heavy metals which appear to be causing respiratory failure. The reduced form of the tocopherol derivative...
could be reoxidized metabolically. It is very well known, indeed, that tocopherol itself and reduced tocopherol derivatives undergo oxidation by reacting with iron (III). Thus, one could conceive of the possibility that the hypothetical metabolite serves as an intermediate carrier for electron transfer from reduced mono- or dithiol sites of enzymes or cofactors to iron containing catalysts, for instance, members of the cytochrome chain, or to other iron containing enzyme sites.

2. An alternate possibility is given by the fact that quinonoid structures readily form reaction products with sulfhydryl groups by simple condensation. Such products are chemically well defined and known, for instance, for cystine and benzoquinone, and for glutathione and menadione. It seems possible that tocopherol metabolites of a quinonoid nature, as well as the other active substances mentioned, have a masking or shielding effect on labile SH groups by virtue of this mechanism. One can envision that such reaction products of quinones with sulfhydryl sites are the truly effective, electron transferring configurations. Their formation and stability would be impaired by heavy metals, on one hand, and enhanced by the active compounds, on the other hand.

It is hoped that further pursuit of the approach described here may lead to the identification of the metabolic function of tocopherol on the molecular level. It is my conviction that tocopherol, in its active form, has a distinct catalytic role in intermediary metabolism. I cannot conceive of tocopherol, a vitamin, simply as of a "policeman" trapping radicals and keeping oxygen molecules in line which stray out of line by accident. The antioxidant function may be strictly coincidental to the true metabolic function of the vitamin.

By way of conclusion, I would like to venture a thought which may sound like heresy to some. In the controversy about "specific metabolic function" vs. "antioxidant activity" we may be dealing with a classic case of a pseudoargument. We should not forget that in some specific instances tocopherol is not an anti-but a potent pro-oxidant. It is possible that peroxides are actually normal products in intermediary metabolism and that they fulfill a perfectly useful function in certain oxidative pathways. If lipid peroxides, for instance of essential fatty acids, would be such short lived, normal intermediates, then the active form of tocopherol could be the catalyst which metabolized them further. There is nothing I know which would preclude such possibilities except for the fact that we are not accustomed to think in these terms. The fact that we cannot demonstrate lipid peroxides in metabolizing systems does not mean very much. Scientists have tried in vain for thirty years to find acetate as a normal intermediate, and yet, as we all know, it is indeed a metabolite of utmost importance. At any rate, in the question of the metabolic functions of vitamin E, we have come to a point where a little bit of concrete, positive evidence may go a long way to clear up existing misinterpretations.

REFERENCES