Effect of Dietary Vitamin C and Vitamin E on Tissue Lipid Peroxidation of Guinea Pigs Fed with Oxidized Oil

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Eighteen male guinea pigs were divided into two groups and fed for 2 weeks with a diet containing 284 ppm of vitamin C and 50 ppm of vitamin E. One group received autoxidized linseed oil (40 mg/animal/day) three times during the feeding. Compared with the animals not given the oxidized oil, the body weight gains, liver ascorbic acid levels and α-tocopherol contents were significantly decreased and liver lipid peroxidation was greatly stimulated in the animals dosed with the oxidized oil. In the next experiment, forty male guinea pigs were divided into five groups; only one group received a diet containing 1000 ppm of vitamin C and 50 ppm of vitamin E without oxidized oil, and the other four groups received graded diets of 1000–3000 ppm of vitamin C and 50–200 ppm of vitamin E for 35 days. To the latter four groups, the oxidized oil (24 mg/animal/day) was orally administered 12 times during the feeding period. The tocopherol contents of the kidney and lung were found to be lower in the animals fed with a 3000 ppm-vitamin C and 200 ppm-vitamin E diet than those fed with a 1000 ppm-vitamin C and 200 ppm-vitamin E diet. The lung ascorbic acid content was lower in animals fed with a 3000 ppm-vitamin C and 200 ppm-vitamin E diet than in those fed with a 3000 ppm-vitamin C and 50 ppm-vitamin E diet. The effect of dietary vitamins C and E on tissue lipid peroxidation was different among the individual organs. From the results obtained by chemiluminescence analyses, the lipid peroxidation caused in the liver, kidney and heart was recognized to be suppressed effectively when 200 ppm-vitamin E diets were given. Antioxidative synergism of vitamin E (200 ppm) and C (3000 ppm) was found in the liver and lung as estimated by the chemiluminescence.

Vitamin C has been shown to be important for the regeneration of vitamin E from the tocopherol radical in in vitro systems, suggesting a synergistic effect with regard to the two vitamin antioxidant activities.1–5) However, the exact nature of the in vivo interaction between vitamins C and E is still not clear. Kunnert and Tappel6) have demonstrated the antioxidative action of ascorbic acid on lipid peroxidation caused by CCl₄-poisoning in guinea pigs bred on a vitamin C-deficient diet, as measured by pentane and ethane production. However, Chen7) has provided evidence that supplementation of high levels of dietary vitamin C leads to erythrocyte hemolysis in rats. We therefore wished to ascertain how the dietary status of vitamin C and vitamin E might affect the tissue lipid peroxidation in guinea pigs fed with an oxidized oil. The oxidized oil intake stimulated lipid peroxidation in several animal tissues.8–11) In this study, the chemiluminescence analysis was mainly used to estimate the degree of tissue lipid peroxidation.12,13) Since guinea pigs do not have the capability for synthesizing ascorbic acid, we chose them as an appropriate animal model for these experiments.

MATERIALS AND METHODS

Male guinea pigs of the Hartley strain (Agricultural Cooperative Association for Laboratory Animals, Hamamatsu) were used throughout the experiments. For a week prior to the experiments, the animals were bred on commercial pellet rations (guinea pigs diet GM-4S containing 1000 ppm of vitamin C and 50 ppm of vitamin E, Funabashi Farm Co., Chiba) to a body weight of 240 g. Each animal was kept in a wire-bottomed stainless-steel cage and water was supplied ad libitum. Room tempera-
ture was kept at 24°C with a 12-hr cycle of light and dark. In the experiments, we adjusted the dietary ascorbic acid levels to 284~3000 ppm and RRR-α-tocopherol (Eisai Co., Tokyo) to 50~200 ppm in a commercial diet free of vitamin C and E purchased from Oriental Yeast Co., Tokyo, in which the nutritional composition was appropriate except for vitamin C and E.

Experiment 1. The guinea pigs were randomly divided into two groups, each group consisting of 9 animals. To both groups, the diet containing 284 ppm of vitamin C and 50 ppm of vitamin E was fed for 2 weeks. The diet was supplied at 25 g/animal/day. The level of 7.1 mg/240 g of body wt./day of vitamin C is threefold the minimum amount to maintain the growth of guinea pigs. To the animals of one group autoxidized linseed oil with a peroxide value of 906 meq/kg and carbonyl value of 480 meq/kg was orally administered (40 mg/animal/day) by stomach tube at 2, 8 and 12 days, respectively, during the feeding period. The animals were then killed under light anesthesia with ether, and the livers perfused with 20 ml of 0.15 M NaCl were obtained.

The hepatic concentration of ascorbic acid was determined by the dinitrophenylhydrazine method, and tocopherol content by the high-performance liquid chromatography method.

The glutathione peroxidase (GSH-Px) activity of the liver was assayed by the DTNB method (5,5'-dithiobis-2-nitrobenzoate; Aldrich Chem. Co.) using 1.5 mM cumene hydroperoxide as the substrate. As reported by Hafeman et al., the storage of liver at -20°C for 4 to 6 days resulted in noticeable loss of GSH-Px activity, so that in these cases only relative values against the control animal were considered.

Chemiluminescence spontaneously emitted from the liver homogenate was measured to study the peroxidation of liver lipids by a method previously reported by Miyazawa et al. After killing the animals, the liver was immediately subjected to chemiluminescence analysis. A synchronous single photon counting apparatus (OX-7C Chemiluminescence Analyzer, Tohoku Electronic Industries Co., Sendai) was used for detecting the light emission. A 5 ml sample of 10% (w/v) liver-physiological saline homogenate was placed on a stainless-steel plate (53 mm in diameter, 13 mm in height) and analyzed under an air atmosphere at 37°C for 5 min. All the procedures for measuring chemiluminescence were performed in the dark. The chemiluminescence intensity is expressed in terms of average counts per 20 sec for the 5-min measurements and is corrected for background counts. The liver TBA (thiobarbituric acid) value, as a convenient index for lipid peroxidation, was determined by the method of Ohkawa et al.

Experiment 2. The guinea pigs were divided into five groups, each group consisting of 8 animals. The animals were fed ad libitum for 35 days with diets supplemented with vitamin C at 1000 ppm and vitamin E at 50 ppm (groups A and B), 3000 ppm of vitamin C and 50 ppm of vitamin E (group C), 1000 ppm of vitamin C and 200 ppm of vitamin E (group D), and 3000 ppm of vitamin C and 200 ppm of vitamin E (group E). During the feeding of groups B, C, D and E, autoxidized linseed oil, the same as that used in Experiment 1, was orally administered (24 mg/animal/day) at 3, 5, 8, 11, 14, 17, 20, 23, 26, 29, 30 and 33 days. Subsequently, the liver, kidney, heart and lung were separated from the animals and the ascorbic acid and α-tocopherol contents, GSH-Px activities, chemiluminescence intensities and TBA values were determined in the same manners as those indicated in Experiment 1. Statistical significance in the difference of values was analyzed using Student’s t-test.

RESULTS

Experiment 1

In this experiment, we studied the effect of feeding a 284 ppm-vitamin C diet on the growth and some characteristics of the liver of guinea pigs exposed to oxidative stress with oxidized oil administration. Table I shows the body weight gain, liver ascorbic acid and α-tocopherol contents, GSH-Px activity, chemiluminescence intensity and TBA value of guinea pigs fed with a 284 ppm-vitamin C and 50 ppm-vitamin E diet for 2 weeks with or without oxidized oil. The body weight gain and liver weight of those animals which received oxidized oil were lower than those not given the oxidized oil. With the feeding of oxidized oil, the liver ascorbic acid and α-tocopherol contents significantly decreased; especially, the ascorbic acid content was reduced to one half the value in animals not receiving oxidized oil. When oxidized oil was given, the liver GSH-Px activity, chemiluminescence intensity and TBA value were considerably more than those of animals not given oxidized oil.

Experiment 2

We conducted this experiment to study the antioxidative effect of increased levels of dietary vitamin C and E on the individual organs of guinea pigs fed with oxidized oil.

Table II shows the effect of oxidized oil administration on the liver of animals bred on diets with 1000~3000 ppm of vitamin C and
Table I. Effect of Oxidized Oil Administration on Guinea Pigs Fed with a 284 ppm-Vitamin C Diet for 14 Days1

<table>
<thead>
<tr>
<th>Observations</th>
<th>Oxidized oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadministered</td>
</tr>
<tr>
<td>Body wt. gain (g/14 days)</td>
<td>55 ± 5</td>
</tr>
<tr>
<td>Liver wt. (% of body wt.)</td>
<td>4.6 ± 0.5</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>ascorbic acid (µg/g of tissue)</td>
<td>98.5 ± 3.3</td>
</tr>
<tr>
<td>α-tocopherol (µg/g of tissue)</td>
<td>4.7 ± 0.9</td>
</tr>
<tr>
<td>glutathione peroxidase (%)3</td>
<td>100 ± 27</td>
</tr>
<tr>
<td>chemiluminescence (counts/20 sec)</td>
<td>81 ± 18</td>
</tr>
<tr>
<td>TBA value (MDA4 nmol/g of tissue)</td>
<td>181 ± 34</td>
</tr>
</tbody>
</table>

1 Mean ± S.D. for 9 animals.
2 Significantly different (p < 0.05) from the oxidized oil-unadministered group.
3 Enzyme activity is expressed as the percentage of that found in the group not receiving oxidized oil.
4 MDA, malondialdehyde.

Effect of Vitamins C and E on Tissue Lipid Peroxidation

50~200 ppm of vitamin E for 35 days. As can be seen in the groups A and B both fed with a diet including 1000 ppm of vitamin C and 50 ppm of vitamin E, the liver tocopherol content was significantly reduced as a result of oxidized oil administration. A large increase was found in the liver chemiluminescence intensity and TBA value in group B, as compared with those in group A. Among the groups administered with oxidized oil, the liver chemiluminescence intensity and TBA value of animals receiving a 200 ppm-vitamin E diet (groups D and E) were lower than those of groups B and C to which a 50 ppm-vitamin E diet was given. The increase of dietary vitamin C level did not cause any decrease in liver chemiluminescence and TBA value as denoted in group C. Liver GSH-Px activity was irrelevant to the oxidized oil treatment.

In the heart (Table IV), the ascorbic acid and α-tocopherol contents were directly affected by the dietary levels of both vitamins. Even after the oxidized oil treatment, no significant change was observed on either vitamin content in the heart. The heart GSH-Px activity was not affected by the oxidized oil treatment and the dietary vitamin levels. The heart chemiluminescence intensity that was enhanced by the oxidized oil administration was less in groups D and E, to which 200 ppm-vitamin E diets were given. The heart TBA value did not show such a significant change as observed with chemiluminescence.

In the lung (Table V), the ascorbic acid content was decreased by the oxidized oil administration, as indicated by the difference between groups A and B, both of which received a 1000 ppm-vitamin C and 50 ppm-vitamin E diet. The lung ascorbic acid content of group E, which was fed with a diet incorporating 3000 ppm of vitamin C and 200 ppm of vitamin E, was lower than in the group C animals fed with a diet including 3000 ppm of vitamin C and 50 ppm of vitamin E. The lung tocopherol content of group E animals fed with a 3000 ppm-vitamin C diet was significantly lower than those of group D fed with a 1000 ppm-vitamin C diet, in which both diets contained 200 ppm of vitamin E. Lung GSH-Px activity was enhanced in the
### Table II. Effect of Oxidized Oil Administration on the Liver of Guinea Pigs Fed with Diets Incorporating Graded Levels of Vitamins C and E for 35 Days

<table>
<thead>
<tr>
<th>Group</th>
<th>Vit. C (ppm)</th>
<th>Vit. E (ppm)</th>
<th>Oxidized oil</th>
<th>Ascorbic acid (µg/g of tissue)</th>
<th>α-Tocopherol (µg/g of tissue)</th>
<th>Glutathione peroxidase (%)*</th>
<th>Chemiluminescence (counts/20 sec)</th>
<th>TBA value (MDA² nmol/g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1000</td>
<td>50</td>
<td>-</td>
<td>145 ± 20abc</td>
<td>7.5 ± 0.9b</td>
<td>100 ± 9b</td>
<td>187 ± 30a</td>
<td>355 ± 47a</td>
</tr>
<tr>
<td>B</td>
<td>1000</td>
<td>50</td>
<td>+³</td>
<td>164 ± 26a</td>
<td>5.7 ± 0.6a</td>
<td>105 ± 11bc</td>
<td>589 ± 68b</td>
<td>598 ± 85b</td>
</tr>
<tr>
<td>C</td>
<td>3000</td>
<td>50</td>
<td>+</td>
<td>271 ± 29b</td>
<td>8.4 ± 1.5b</td>
<td>124 ± 11c</td>
<td>452 ± 71c</td>
<td>697 ± 88b</td>
</tr>
<tr>
<td>D</td>
<td>1000</td>
<td>200</td>
<td>+</td>
<td>139 ± 21a</td>
<td>16.5 ± 2.1c</td>
<td>89 ± 17bc</td>
<td>290 ± 38b</td>
<td>418 ± 66b</td>
</tr>
<tr>
<td>E</td>
<td>3000</td>
<td>200</td>
<td>+</td>
<td>289 ± 14b</td>
<td>15.9 ± 1.2c</td>
<td>78 ± 9a</td>
<td>212 ± 26a</td>
<td>368 ± 74a</td>
</tr>
</tbody>
</table>

1. Data is presented as the mean ± SD for 8 guinea pigs.
2. Enzyme activity is expressed as the percentage of that found in group A.
3. Animals of group B, C, D and E were orally administered with oxidized oil.
4. Means not followed by a common letter are significantly different (p < 0.05).
5. MDA, malondialdehyde.

### Table III. Effect of Oxidized Oil Administration on the Kidney of Guinea Pigs Fed with Diets Incorporating Graded Levels of Vitamins C and E for 35 Days

<table>
<thead>
<tr>
<th>Group</th>
<th>Vit. C (ppm)</th>
<th>Vit. E (ppm)</th>
<th>Oxidized oil</th>
<th>Ascorbic acid (µg/g of tissue)</th>
<th>α-Tocopherol (µg/g of tissue)</th>
<th>Glutathione peroxidase (%)*</th>
<th>Chemiluminescence (counts/20 sec)</th>
<th>TBA value (MDA² nmol/g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1000</td>
<td>50</td>
<td>-</td>
<td>129 ± 12abc</td>
<td>3.2 ± 0.3a</td>
<td>100 ± 11b</td>
<td>168 ± 15a</td>
<td>273 ± 20a</td>
</tr>
<tr>
<td>B</td>
<td>1000</td>
<td>50</td>
<td>+³</td>
<td>141 ± 12a</td>
<td>2.9 ± 0.3a</td>
<td>76 ± 26b</td>
<td>395 ± 81b</td>
<td>348 ± 54b</td>
</tr>
<tr>
<td>C</td>
<td>3000</td>
<td>50</td>
<td>+</td>
<td>177 ± 9b</td>
<td>3.4 ± 0.4a</td>
<td>55 ± 17b</td>
<td>190 ± 29b</td>
<td>359 ± 27b</td>
</tr>
<tr>
<td>D</td>
<td>1000</td>
<td>200</td>
<td>+</td>
<td>144 ± 12a</td>
<td>8.7 ± 1.4c</td>
<td>57 ± 16a</td>
<td>152 ± 15a</td>
<td>342 ± 47b</td>
</tr>
<tr>
<td>E</td>
<td>3000</td>
<td>200</td>
<td>+</td>
<td>168 ± 9b</td>
<td>5.3 ± 0.5b</td>
<td>100 ± 12b</td>
<td>195 ± 41a</td>
<td>355 ± 34b</td>
</tr>
</tbody>
</table>

Descriptions are the same as those in the legend to Table II.
### Table IV. Effect of Oxidized Oil Administration on the Heart of Guinea Pigs Fed with Diets Incorporating Graded Levels of Vitamins C and E for 35 Days

<table>
<thead>
<tr>
<th>Group</th>
<th>Vit. C (ppm)</th>
<th>Vit. E (ppm)</th>
<th>Oxidized oil</th>
<th>Ascorbic acid (µg/g of tissue)</th>
<th>α-Tocopherol (µg/g of tissue)</th>
<th>Glutathione peroxidase (V)²</th>
<th>Chemiluminescence (counts/20 sec)</th>
<th>TBA value (MDA² nmol/g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1000</td>
<td>50</td>
<td>−</td>
<td>105 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.3 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100 ± 12&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>102 ± 9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>232 ± 25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>1000</td>
<td>50</td>
<td>+&lt;sup&gt;3&lt;/sup&gt;</td>
<td>90 ± 10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.9 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106 ± 30&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>145 ± 10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>265 ± 28&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>3000</td>
<td>50</td>
<td>+</td>
<td>120 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.8 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100 ± 18&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>155 ± 17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>225 ± 25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>1000</td>
<td>200</td>
<td>+</td>
<td>105 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.2 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>127 ± 36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>112 ± 10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>250 ± 25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>E</td>
<td>3000</td>
<td>200</td>
<td>+</td>
<td>138 ± 10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.5 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80 ± 15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106 ± 17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>230 ± 20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Descriptions are the same as those in the legend to Table II.

### Table V. Effect of Oxidized Oil Administration on the Lung of Guinea Pigs Fed with Diets Incorporating Graded Levels of Vitamins C and E for 35 Days

<table>
<thead>
<tr>
<th>Group</th>
<th>Vit. C (ppm)</th>
<th>Vit. E (ppm)</th>
<th>Oxidized oil</th>
<th>Ascorbic acid (µg/g of tissue)</th>
<th>α-Tocopherol (µg/g of tissue)</th>
<th>Glutathione peroxidase (V)²</th>
<th>Chemiluminescence (counts/20 sec)</th>
<th>TBA value (MDA² nmol/g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1000</td>
<td>50</td>
<td>−</td>
<td>189 ± 10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.8 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100 ± 18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58 ± 9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>128 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>1000</td>
<td>50</td>
<td>+&lt;sup&gt;3&lt;/sup&gt;</td>
<td>148 ± 14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.3 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>171 ± 30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95 ± 17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>148 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>3000</td>
<td>50</td>
<td>+</td>
<td>240 ± 25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.0 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>236 ± 46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>89 ± 16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>145 ± 10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>1000</td>
<td>200</td>
<td>+</td>
<td>179 ± 31&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>28.0 ± 2.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>213 ± 41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71 ± 8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>148 ± 9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>E</td>
<td>3000</td>
<td>200</td>
<td>+</td>
<td>170 ± 18&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>19.5 ± 3.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>230 ± 64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54 ± 12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>118 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Descriptions are the same as those in the legend to Table II.
oxidized oil-administered animals. The lowest chemiluminescence intensities and TBA values were found in group E.

DISCUSSION

Oxidized oil intake causes tissue lipid peroxidation and has been reported to increase the chemiluminescence intensity and TBA value of such internal organs as the liver, kidney, heart, lung and brain in rats. The large enhancement of the lipid peroxidation-supported chemiluminescence of tissue homogenates of rats caused by the long-term administration of methyl linoleate hydroperoxides was shown to be prevented by dietary antioxidants such as α-tocopherol and riboflavin-butyrate.

The mechanism of hydroperoxide-induced tissue chemiluminescence has been explained by a hydroperoxide disproportionation reaction, and directly indicates the generation of electrically excited species during the lipid peroxidation. Singlet oxygen and triplet carbonyl compounds were shown to participate as the major emission species in the chemiluminescence of liver homogenates supplemented in vitro with oxidized lipids, and the liver of animals administered with autooxidized linseed oil. Therefore, it could be said that the increase of tissue chemiluminescence reflects the burst progression of oxidative free radical events involved in the lipid peroxidation reaction. The lipid peroxy radical, which is responsible for chemiluminescence, has been identified in vitro and in vivo systems by us with an ESR study. On the other hand, a TBA assay is believed to measure the amount of secondary products of lipid peroxidation, such as conjugated aldehydes and endoperoxides including prostaglandins, rather than measuring the lipid hydroperoxides directly. The reactivity of TBA reagent with lipid hydroperoxides has been reported to be below 10%. The chemiluminescence and TBA value are considered to reflect different stages in tissue lipid peroxidation as depicted in the following scheme:

ROOH (lipid hydroperoxides)

\[
\begin{align*}
\text{ROO}^- & \rightarrow 1\text{O}_2^- + \text{RC}=\text{O}^* \rightarrow \text{chemiluminescence} \\
\text{RO}^- & \rightarrow \text{(singlet (triplet}} \\
\text{R}^- & \rightarrow \text{oxygen) carbonyls) \\
\text{secondary products} & \rightarrow \text{TBA reactants} \\
\text{(aldehydes and endoperoxides)} &
\end{align*}
\]

The GSH-Px of the liver in guinea pigs has been shown to be mostly the non selenium-dependent enzyme, in contrast to the GSH-Px in rats. In rats and mice, the GSH-Px activity of the liver and small intestine was enhanced by long-term feeding with oxidized lipids.

The results (Table I) obtained in Experiment 1 indicate that lipid peroxidation of the liver was also significantly stimulated in guinea pigs by feeding with oxidized oil. The progress of liver lipid peroxidation was recognized by the large increase of chemiluminescence and TBA values (Table I). The enhanced GSH-Px activity of liver in the oxidized oil-dosed animal (Table I) may also have resulted from the progression of hepatic lipid peroxidation.

As shown in Table I, the large decrease in liver ascorbic acid content with the increased hepatic lipid peroxidation resulting from feeding with oxidized oil in guinea pigs indicates that the liver ascorbic acid was consumed during lipid peroxidation. From the point of view that vitamin C is related to the regeneration of vitamin E, a potent bioantioxidant, from the tocopherol radical, it is an interesting finding that the liver tocopherol level was reduced together with the
decrease of liver ascorbic acid content in the oxidized oil-dosed animals fed with a 284 ppm-vitamin C and 50 ppm-vitamin E diet (Table I).

The data given in Table II show that, even in animals receiving 1000 ppm and 3000 ppm of dietary vitamin C, respectively corresponding to nine and twenty seven times the minimum amount to maintain the growth of guinea pigs,31) liver lipid peroxidation was caused by the administration of oxidized oil. The liver lipid peroxidation is then considered to have been prevented by the 200 ppm-vitamin E supplement but not by 50 ppm of vitamin E (Table II). A synergistic antioxidative effect with vitamins E and C was observed in the liver chemiluminescence when the dietary vitamin E and C levels were increased to 200 ppm and 3000 ppm, respectively (Table II).

When the liver tocopherol and ascorbate levels were reduced in guinea pigs by feeding with a tocopherol- and ascorbate-free diet for 3 weeks, Chen and Chang29) have shown that vitamin C supplementation (2 mg/100 g body wt./day) reduced the liver lipid peroxidation caused by these vitamin deficiencies. The inconsistency between our present results in Table II and the findings by Chen and Chang29) would be due to the difference in the method to cause tissue lipid peroxidation. Almost the same thing could be said against the data reported by Kunnert and Tappel,6) who used ascorbate-deficient animals with poisoning of CCl₄ as a model for in vivo lipid peroxidation. In our present experiments, an oxidized oil-induced lipid peroxidation system was employed as a model for studying the tissue lipid peroxidation that even occurred under a more physiologically normal condition than those in a model with a tocopherol and/or ascorbate deficiency. The reduced lipid peroxidation observed by supplementing tocopherol and/or ascorbate to the tocopherol and/or ascorbate deficient animals as have been reported by these investigators6,29) would not be always ascribable to the antioxidant action with these vitamins.

The data shown in Table III suggest that either the 3000 ppm-vitamin C or 200 ppm-vitamin E dists prevented kidney lipid peroxidation, as indicated by the chemiluminescence. It was also found that high vitamin C (3000 ppm) supplementation caused a decrease in the kidney tocopherol content, compared with the case of a lower vitamin C (1000 ppm) supplement (Table III). This may imply a direct interaction between the tocopheryl radical and ascorbate in the kidney as suggested in in vitro systems.1~4) The inconsistency found in the kidney chemiluminescence and TBA value in groups C, D and E (Table III) may due to the different stage of lipid peroxidation being monitorable by both indices as already mentioned before. The precise reason for this inconsistency has not yet been found.

A large supplement of vitamin E (200 ppm) seems to decrease lipid peroxidation of the heart, as judged by the chemiluminescence (Table IV). If the breakdown products of lipid peroxides were efficiently metabolized even under the progress of lipid peroxidation in the heart, the TBA value would be lower and the chemiluminescence would be higher, as observed in the heart of groups B and C (Table IV).

In the lung (Table V), a synergistic antioxidative effect with dietary vitamins E (200 ppm) and C (3000 ppm) was found by the chemiluminescence and TBA value as considered in group E.

From the results obtained in Experiment 2, the effect of dietary vitamin C and E in guinea pigs fed with an oxidized oil was seen to be different among the individual organs. Namely, the lipid peroxidation as measured by chemiluminescence was reduced in the liver and heart by a high level of vitamin E (200 ppm) in the diets, while in the kidney, the lipid peroxidation was suppressed by both a high vitamin C (3000 ppm) diet and high vitamin E (200 ppm) diet. An antioxidative synergism of vitamin E (200 ppm) and C (3000 ppm) was observed in the liver and lung, as expressed by the chemiluminescence analysis. It can be said from the data in Experiment 1 that vitamin C and E are actually consumed on the occasion of hepatic lipid peroxidation in guinea pigs. But it seems
unlikely that there is an antioxidative function with large doses of vitamin C only, as revealed in the tissue organs except for the kidney in Experiment 2. The supplement of vitamin E in the diet was found to be more pertinent to prevent the tissue lipid peroxidation caused by an oxidized oil dose in guinea pigs, rather than the large supplement of vitamin C.

REFERENCES