Vitamin E and stress

7. The interrelationships between polyunsaturated fatty acid stress, vitamin A and vitamin E in the rat and the chick

BY J. GREEN, I. R. MUTHY, A. T. DIPLOCK, J. BUNYAN, M. A. CAWTHORNE AND ELSPETH A. MURRELL

Walton Oaks Experimental Station, Vitamins Ltd, Tadworth, Surrey

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1. The nature of the relationship between vitamins A and E has been studied in the rat and the chick.
2. Stress induced by diets rich in polyunsaturated fatty acids (PUFA) was found to have no effect on the liver vitamin A reserves of vitamin E-deficient rats given dietary vitamin A or repeated small oral doses of vitamin A.
3. Dietary PUFA did not affect the liver vitamin A reserves of young rats given necrogenic diets deficient in vitamin E and selenium, nor were these reserves affected by the onset of liver necrosis or its prevention by Se.
4. The effect of dietary PUFA on the rate of depletion of liver vitamin A reserves in weanling rats or rats depleted initially of vitamins A and E and then given a single large dose of vitamin A was studied over periods from 2 to 12+ weeks. In three experiments the dietary PUFA did not significantly accelerate vitamin A depletion. In one experiment the depletion rate was increased, but this was not reversed by dietary vitamin E and thus could not be attributed to an enhancement of peroxidation in vivo but rather to a toxic effect. The effect of vitamin E in these experiments was not consistent but, in general, it slightly decreased the rate of depletion.
5. Large doses of vitamin A did not affect the metabolism of small amounts of [14C]p-α-tocopherol in the vitamin E-deficient rat or chick, when interaction of the two vitamins in the gastro-intestinal tract was avoided.
6. Large doses of vitamin A (40000 i.u. in total) given to vitamin E-deficient chicks receiving a diet containing 1% linoleic acid (as maize oil esters) did not accelerate the onset of encephalomalacia and therefore failed to exert a pro-oxidative effect on tissue tocopherol.
7. The conclusion drawn from these experiments was that any relationship that may exist in vivo between vitamins A and E is not concerned with an effect of vitamin E in preventing oxidation of vitamin A. A critical review of the literature on the nature of the relationship in general supports this view.

It has been known for many years that a nutritional relationship exists between vitamins A and E, and the nature of this relationship has been the subject of many investigations. Moore (1940) originally put forward the view that vitamin E might influence the depletion rate of vitamin A by affecting liver function; but Davies & Moore (1941), who found that vitamin E markedly increased vitamin A concentrations in rat liver, suggested that tocopherol, by virtue of its antioxidant properties, protected vitamin A against oxidation in vivo. Since then, much further work has been done on the problem and has generally appeared to support the views of Davies & Moore (1941). Although most workers have agreed on the existence of the nutritional relationship, there has been considerable disagreement as to whether it is due essentially to an interaction between the two vitamins in the intestinal tract before absorption or in the tissues of the animal after absorption. In recent years, the function of vitamin E has become identified with that of a 'biological antioxidant' and the distinction

between these two modes of action has been blurred. Thus Tappel (1962), in his review of the biological antioxidant theory, regards all vitamin E effects as protecting against the proliferation of 'lipid peroxidation' in vivo and regards the vitamin A–vitamin E relationship as a subsidiary part of that. In essence, therefore, vitamin A is usually regarded as a 'co-oxidizable' substrate, the destruction of which can be decreased in vivo by the antioxidant action of vitamin E.

In several earlier papers, however, we found no evidence for enhanced lipid peroxidation in the chick or the rat in the absence of vitamin E or selenium, nor did we consider that these two substances functioned as antioxidants in vivo (Green, Diplock, Bunyan, McHale & Muthy, 1967; Diplock, Green, Bunyan, McHale & Muthy, 1967; Bunyan, Murrell, Green & Diplock, 1967). If, therefore, a biochemical relationship exists in vivo between vitamins A and E, it would now not seem possible to attribute it to that between an oxidizable substrate and an antioxidant. We have therefore reinvestigated the problem from several new points of view.

**EXPERIMENTAL AND RESULTS**

**Materials and methods**

*Animals.* Norwegian hooded rats of both sexes were given the vitamin E-deficient diet A 10 Y 3 (Bunyan, McHale & Green, 1963) or diet A 3 (Bunyan, Green, Diplock & Robinson, 1967a) until the start of each experiment. The diets used in the various experiments are described in the text. The rats were allocated to experimental groups at random with litter-mate controls and, where appropriate, with equal distribution of sexes. In the experiment with radioactive \( \alpha \)-tocopherol, the rats were individually housed in tubular cages to prevent coprophagy (Green, Diplock, Bunyan, McHale & Muthy, 1967). Chicks were 1-day-old Warren cockerels, purchased from a commercial hatchery, and reared on wire floors in electrically heated cages. Their experimental diets are described in the text.

*Materials.* Methyl oleate (OLME), maize oil methyl esters (MOME), cod-liver oil methyl esters (CLOME) and [\(^{14}\)C]\(\alpha\)-tocopherol were prepared as described by Green, Diplock, Bunyan, McHale & Muthy (1967). [\(\alpha\)-\(^{14}\)C]Retinyl acetate was obtained from the Radiochemical Centre, Amersham.

*Vitamin A analyses.* In Expts 1, 2, 3, 5, 9 and 10 these were carried out on a portion of the non-saponifiable extract obtained from liver or from whole carcass by the methods described by Green, Diplock, Bunyan, McHale & Muthy (1967). A suitable volume of the extract in ether was evaporated to dryness, and vitamin A was measured spectrophotometrically at 620 nm after reaction with antimony trichloride in the usual way. In Expts 4, 6, 7 and 8, vitamin A was determined by the method of Ames, Risley & Harris (1954).

*Polyunsaturated fatty acid (PUFA) analysis.* This was carried out by the enzymic method of MacGee (1959).

*Radioactivity measurements.* Analyses for [\(^{14}\)C]\(\alpha\)-tocopherol and its fat-soluble metabolites were as described by Green, Diplock, Bunyan, McHale & Muthy (1967).
Polyunsaturated fatty acid stress and the vitamin A reserves of vitamin E-deficient rats given dietary vitamin A or repeated small doses of vitamin A

We have argued previously (Green, Diplock, Bunyan, McHale & Muthy, 1967) that if ‘anti-vitamin E’ stress involves an increase of lipid peroxidation in vivo, it must result in a loss of so-called ‘tissue antioxidants’. The same principle must apply also to the postulated phenomenon of ‘co-oxidation’. The point is put clearly by Tappel (1962): ‘haematin-catalysed lipid peroxidation can be very damaging to other biological compounds in its proximity because the free radicals will react at random through hydrogen abstraction and a variety of addition reactions. Oxygen-labile compounds like vitamin A are readily co-oxidized. Present knowledge indicates that vitamin A is co-oxidized in vivo in a haemoglobin-catalysed lipid peroxidation.’ If this is so, an increase in tissue ‘peroxidizability’ would be expected to result in increased vitamin A destruction in vivo. Since tissue ‘peroxidizability’ is readily increased by increasing the ‘peroxidizability’ of the dietary lipid (Witting, 1965a, b; Witting & Horwitt, 1964; Witting, Harmon & Horwitt, 1965) the point can be verified experimentally.

Expt 1. In this experiment the effect of a moderate amount of dietary PUFA was studied. Sixteen 4-month-old vitamin E-deficient male rats were divided into two groups. One group was given diet \(a_3\) (containing 3\% lard and 11 i.u. vitamin A/g) with 5\% OLME added weekly and the other group diet \(a_3\) with 5\% CLOME added daily. The additives replaced 2\% sugar and 3\% lard. After 21 days the rats were killed and their livers were analysed for vitamin A. The results are shown in Table 1. There was no significant difference between the vitamin A contents of the two groups, even though, in this experiment, the dietary lipid was allowed to interact with vitamin A in the intestinal tract.

Expt 2. In this experiment the effect of a large amount of dietary lipid was studied. Twelve 4-month-old vitamin E-deficient male rats were divided into two groups. One group received diet \(a_3\) with 20\% OLME added weekly, the other the diet with 20\% CLOME added daily, the additives replacing 17\% sugar and 3\% lard. After 16 days the rats were killed and their livers were analysed for vitamin A. The results are given in Table 1. The vitamin A content of the livers was unaffected by the severe dietary stress (post-mortem examination revealed considerable gastro-intestinal inflammation in the rats given CLOME). In this experiment also the dietary lipid was allowed to react with vitamin A in the intestinal tract. Peroxidative destruction of vitamin A in the gut of rats given CLOME would be expected to be greater than in rats given OLME, but the effect may have been counterbalanced by increased absorption in the presence of dietary PUFA (cf. Expt 7, this paper, and Week & Sevigne (1949a, b), who found that dietary maize oil doubled the efficiency of vitamin A absorption in rats, compared to controls given ethyl laurate).

Expt 3. In this experiment the effect of lipid on the absorption process was eliminated, and a study was made of the effect of dietary PUFA on the depletion of vitamin A from weanling rats given a vitamin E-deficient diet. Twelve weanling rats of both sexes at 30 days of age were divided into two groups, to receive the vitamin A-
Table 1. Expts 1, 2 and 3. Effect of polyunsaturated fatty acid stress on the vitamin A reserves of rats given vitamin E-deficient diets with continuous or repeated doses of vitamin A

(In each experiment, one group of rats was given dietary methyl oleate (OLME) and the other dietary cod-liver oil methyl esters (CLOME). The rat livers were combined in pairs for analysis. Results are given as means with standard deviations.)

<table>
<thead>
<tr>
<th>Expt</th>
<th>Vitamin A administration</th>
<th>Experimental period (days)</th>
<th>Age of rats (months)</th>
<th>No. of rats/group</th>
<th>Dietary lipid content (%)</th>
<th>Rat wt at death OLME group (g)</th>
<th>CLOME group (g)</th>
<th>Vitamin A OLME group (i.u./liver)</th>
<th>CLOME group (i.u./liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11 i.u./g diet</td>
<td>21</td>
<td>4</td>
<td>8</td>
<td>5</td>
<td>163 ± 21</td>
<td>164 ± 21</td>
<td>1325 ± 122</td>
<td>1782 ± 513</td>
</tr>
<tr>
<td>2</td>
<td>11 i.u./g diet</td>
<td>16</td>
<td>4</td>
<td>6</td>
<td>20</td>
<td>217 ± 31</td>
<td>200 ± 24</td>
<td>2685 ± 607</td>
<td>2629 ± 349</td>
</tr>
<tr>
<td>3</td>
<td>Single doses of 100 i.u./week</td>
<td>22</td>
<td>1</td>
<td>6</td>
<td>10</td>
<td>63 ± 6</td>
<td>72 ± 6</td>
<td>20.7 ± 5.1</td>
<td>36.7 ± 6.2*</td>
</tr>
</tbody>
</table>

* Significantly higher than value for OLME group (P < 0.05).
free 20% casein diet described by Green, Diplock, Bunyan, McHale & Muthy (1967) supplemented with 0.4% MOME. The diet of one group was supplemented weekly with 10% OLME and the other daily with 10% CLOME, both replacing sugar. Each rat was also given 1000 i.u. vitamin A per week by mouth. After 22 days they were killed and their livers were analysed for vitamin A. The results are given in Table 1. Significantly more vitamin A was found in the livers of the rats given CLOME.

The effect of polyunsaturated fatty acid stress on vitamin A in young rats given a necrogenic diet deficient in vitamin E and Se

The withdrawal of Se from the diet of the young vitamin E-deficient rat leads to the rapid development of liver necrosis. If this is the result, as has been postulated (Caldwell & Tappel, 1964; Century & Horwitt, 1965), of an increase in lipid peroxidation in vivo, it would be expected that the development of necrosis would be accompanied by an increased destruction of vitamin A in the liver. This point was examined in the two following experiments.

Expt 4. Weanling rats of both sexes, weighing 30-50 g at 32 days of age, were each given a single oral dose of 1000 i.u. vitamin A palmitate. After 24 h, they were allocated to two groups. One group received a torula yeast diet supplemented weekly with 5% OLME and the other group the diet supplemented daily with 5% CLOME, both additives replacing sugar. The torula yeast diet, which contained no vitamin A, had the percentage composition: torula yeast (Lake States Yeast and Chemical Division of St Regis Paper Co., Rhinelander, Wisconsin, USA) 30, sugar 48.4, glucose 18, salt mixture (Bunyan, Green, Diplock & Robinson, 1967b) 3-2, vitamin mixture (Diplock, Green et al. 1967) 0.4. The diets were stored at 4°C and that containing CLOME was kept under N2. During the next 8 days some rats died with liver necrosis. The survivors were killed on the 10th day and their livers were assayed individually. Results are shown in Table 2. Dietary PUFA did not affect the vitamin A contents of the livers.

Table 2. Expt 4. Effect of dietary lipid on the vitamin A content of livers of weanling rats given a torula yeast necrogenic diet

(Weanling rats (30-50 g) were given 1000 i.u. vitamin A and then a diet containing either 5% methyl oleate (OLME) or 5% cod-liver oil methyl esters (CLOME). Survivors were killed after 10 days and their livers were assayed individually. Results are given as means with standard deviations)

<table>
<thead>
<tr>
<th>Dietary lipid</th>
<th>Incidence of death*</th>
<th>Day of death</th>
<th>Liver wt† (g)</th>
<th>Vitamin A† (i.u./liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLME</td>
<td>2/9</td>
<td>7, 8</td>
<td>2.15 ± 0.23</td>
<td>575 ± 139</td>
</tr>
<tr>
<td>CLOME</td>
<td>4/9</td>
<td>4, 6, 7, 7</td>
<td>2.06 ± 0.14</td>
<td>563 ± 184</td>
</tr>
</tbody>
</table>

* Number of deaths in numerator; no. of rats in group in denominator.
† In survivors.

Expt 5. Weanling rats at 37 days of age, weighing 27-56 g, were allocated to six groups, each of six rats. The basal necrogenic diet for this experiment had the percentage composition: casein (‘vitamin-free’; Nutritional Biochemicals Corp., Cleve-
land, Ohio, USA) 10, salt mixture (Diplock, Green et al. 1967) 5·33, vitamin mixture (Bunyan, Green et al. 1967b) 0·4, sugar 64·3 and glucose 20. This diet contained 11 i.u. vitamin A/g. The groups received the diet with the following additions, made by replacing sugar where necessary; group 1, none; group 2, 0·05 ppm Se (as sodium selenite); group 3, 2% OLME; group 4, 2% OLME and 0·05 ppm Se (as sodium selenite); group 5, 2% MOME; group 6, 2% MOME and 0·05 ppm Se (as sodium selenite). The diets of the latter two groups were made freshly each day. After 11 days the rats were killed and their livers were analysed for vitamin A. In order to demonstrate the necrogenic properties of the diets a parallel test was carried out with four groups of seven to sixteen rats given the diets of groups 1, 3, 5 and 6. The incidence of liver necrosis was observed during 41 days, after which the survivors were killed and examined. The results of both parts of the experiment are given in Table 3. In the survival part of the experiment, there was a low incidence of necrosis in group 1, given the fat-free diet, and none in group 3, given 2% OLME. Green, Diplock, Bunyan, Muthy & McHale (1967) have discussed the low incidence of necrosis in rats given this fat-free casein diet. The addition of 2% MOME to the diet, however, appreciably increased the incidence of liver necrosis, and this was completely prevented by Se. Despite the differences in the incidence of necrosis in these groups, there were no significant differences in the vitamin A contents of the livers.

Table 3. Expt 5. Vitamin A content of livers of young rats given a fat-free necrogenic diet and the effect of dietary supplementation with methyl oleate (OLME), maize oil methyl esters (MOME) and selenium

(Rats at 37 days of age were given the casein diet, which contained 11 i.u. vitamin A/g, with the various supplements. Their livers were analysed after 11 days. Livers were combined in pairs for each analysis (three analyses per group). Results are given as means with standard deviations)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dietary supplement</th>
<th>Incidence of necrosis*</th>
<th>Liver wt (g)</th>
<th>Liver vitamin A (i.u./g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>3/15</td>
<td>4·2 ± 0·7</td>
<td>162 ± 38</td>
</tr>
<tr>
<td>2</td>
<td>0·05 ppm Se</td>
<td>—</td>
<td>4·5 ± 0·6</td>
<td>203 ± 29</td>
</tr>
<tr>
<td>3</td>
<td>2% OLME</td>
<td>0/8</td>
<td>4·2 ± 0·5</td>
<td>193 ± 41</td>
</tr>
<tr>
<td>4</td>
<td>2% OLME + 0·05 ppm Se</td>
<td>—</td>
<td>4·2 ± 0·6</td>
<td>199 ± 49</td>
</tr>
<tr>
<td>5</td>
<td>2% MOME</td>
<td>7/16</td>
<td>3·9 ± 0·9</td>
<td>184 ± 41</td>
</tr>
<tr>
<td>6</td>
<td>2% MOME + 0·05 ppm Se</td>
<td>0/7</td>
<td>4·5 ± 0·4</td>
<td>165 ± 23</td>
</tr>
</tbody>
</table>

* No. of rats in group in denominator; no. with necrosis in numerator.

The effects of vitamin E and polyunsaturated fatty acids on the depletion of the vitamin A reserves of rats given a single large dose of vitamin A

The basal diet for these experiments was the fat-free, vitamin A-free, 20% casein diet described by Green, Diplock, Bunyan, McHale & Muthy (1967). Supplements were made by replacing sugar. Experimental diets containing lipids were made weekly and stored at 4°C. Unconsumed diet was discarded from the rats' food troughs daily.

Expt 6. Weanling rats were each given 2000 i.u. vitamin A by mouth and then divided into four groups to receive the basal diet supplemented with, respectively, 10% OLME (two groups) and 10% CLOME (two groups). One group of rats given...
Table 4. Expts 6 and 7. Effects of polyunsaturated fatty acids and vitamin E on the rate of depletion of the vitamin A reserves of rats given a single large dose of vitamin A

<table>
<thead>
<tr>
<th>Expt</th>
<th>Test dose of vitamin A (i.u.)</th>
<th>Age when dosed (weeks)</th>
<th>Age when killed (weeks)</th>
<th>Vitamin A (i.u./liver)</th>
<th>10% OLME* vitamin E†</th>
<th>10% CLOME† vitamin E†</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>2000</td>
<td>4</td>
<td>6</td>
<td>480 ± 81 (6)</td>
<td>520 ± 170 (6)</td>
<td>540 ± 206 (6)</td>
</tr>
<tr>
<td>7</td>
<td>9550</td>
<td>10</td>
<td>14½</td>
<td>3000 ± 750 (6)</td>
<td>3380 ± 1290 (6)§</td>
<td>2480 ± 620 (6)</td>
</tr>
<tr>
<td></td>
<td>9550</td>
<td>10</td>
<td>22½</td>
<td>1270 ± 810 (5)</td>
<td>1370 ± 400 (6)</td>
<td>1240 ± 570 (6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14½</td>
<td>14½†</td>
<td>3110 ± 390 (5)</td>
<td>3240 ± 400 (6)</td>
<td>3410 ± 580 (5)¶</td>
</tr>
</tbody>
</table>

* Methyl oleate.
† p-α-tocopheryl acetate: 35 mg/rat per week in Expt 6; 120 ppm of the diet in Expt 7.
‡ Cod-liver oil methyl esters.
§ Results for the twelve rats given vitamin E were greater than for the controls (P < 0.05).
¶ Killed 24 h after the dose.
†† Results for the twelve rats given CLOME were greater than for those given OLME (P < 0.05).
OLME and one group given CLOME also received, by mouth, 35 mg D-α-tocopheryl acetate/rat per week. After 14 days the rats were killed and their livers were analysed for vitamin A. The results are shown in Table 4. The various lipids given did not significantly affect the rats’ liver reserves of vitamin A.

Expt 7. In this experiment the initial dose of vitamin A was given to rats whose reserves of vitamin E and vitamin A had already been greatly depleted, in order to decrease the rat-to-rat variation found in Expt 6 and to accentuate any possible effect of the lipids given. Seventy-two rats were given diet A10 Y3 from which the vitamin A was omitted and also a maintenance dose of 15 i.u. vitamin A/rat per week from 2 to 10 weeks of age, when they were allocated to twelve groups of six. One other rat, treated similarly, was found to have only 10 i.u. vitamin A in its liver. Eight groups were given 9550 i.u. vitamin A/rat by mouth and then given, the next day, the four experimental diets (two groups per diet), and the maintenance dose of vitamin A was stopped. These four diets consisted of the basal 20% casein diet with supplements of, respectively, 10% OLME, 10% OLME and 120 ppm D-α-tocopheryl acetate, 10% CLOME, and 10% CLOME and 120 ppm D-α-tocopheryl acetate. One of the two groups of rats given each diet was killed 4½ weeks later and the other group 12½ weeks later. The livers were analysed for vitamin A. As shown in Table 4, the nature of the dietary lipid had no effect on the vitamin A reserves of the rats killed 4½ weeks after the test dose of vitamin A. However, the rats given vitamin E had significantly more vitamin A in their livers than the others. Rats killed 12½ weeks after the test dose did not show this difference. Some other results for the rats killed after 12½ weeks have already been published by Bunyan, Murrell et al. 1967, who found that CLOME induced small increases in the PUFA content of liver and leg muscle and a threefold rise in the PUFA of adipose tissue.

In addition to the possibility that the rate at which vitamin A is depleted from the body by oxidative destruction might be affected by the degree of lipid unsaturation in vivo, different lipids might also influence the ability of the liver to retain vitamin A, in the physiological sense. This may be a factor in experiments in which vitamin A is given continuously, but it would also be confounded with any additional effects of lipids on the absorption of the vitamin and so could not be measured separately. However, the overall effects of OLME, CLOME and vitamin E on the net short-term retention of a test dose of vitamin A were measured in the four remaining groups of rats. These rats had received the four experimental diets given to the other eight groups and also a maintenance dose of 30 i.u. vitamin A/rat per week, from 10 to 14½ weeks of age. They were then given 9550 i.u. vitamin A and killed 24 h later, and their livers were analysed for vitamin A. The results of this part of the experiment are also shown in Table 4. CLOME significantly increased the net retention of vitamin A during the 24 h after the test dose, but vitamin E did not.

Expt 8. Because of the considerable variance among the replicate results of Expt 7, it was decided to repeat the longer-term part of it, using groups of sixteen rather than six rats. Moore (1940) also encountered a great deal of variability in the liver reserves of rats. It is known that rats vary in the time taken to reach the growth plateau characteristic of vitamin A deficiency; they probably also vary in the rate at which they
lose their reserves. In this experiment, in addition to the four groups of rats given the diets of Expt 7, two other groups were given diets supplemented with 5% lard and with 5% lard together with 120 ppm d-α-tocopheryl acetate respectively so as to compare results more closely with those of Moore (1940) who also used diets containing lard. It was also thought possible that vitamin A might take part in a recycling process between the tissues and the lumen of the gut, similar to that found for vitamin E (Green, Diplock, Bunyan, McHale & Muthy, 1967). If so, the vitamin A concentration in liver might be additionally influenced by the nature of the lipids in the gut and vitamin A could possibly be preserved from oxidation during recycling by antioxidants in the gut contents. It was decided to try, therefore, the effects of giving rats CLOME together with the antioxidant IONOX 330 (2,4,6-tri-(3',5'-di-tert.-butyl-4'-hydroxybenzyl)mesitylene; Shell Ltd); which has been reported by Wright,

Table 5. Expt 8. Effects of polyunsaturated fatty acids, vitamin E, and IONOX 330* on the rate of depletion of the vitamin A reserves of rats given a single dose of vitamin A

<table>
<thead>
<tr>
<th>Dietary lipid</th>
<th>Vitamin A (i.u./liver)</th>
<th>PUFA† (mg/g)</th>
<th>Liver</th>
<th>Adipose tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% OLME†</td>
<td>1670 ± 560</td>
<td>10.6</td>
<td></td>
<td>29.1</td>
</tr>
<tr>
<td>5% OLME† + vitamin E§</td>
<td>2130 ± 410</td>
<td>10.3</td>
<td></td>
<td>33.0</td>
</tr>
<tr>
<td>5% CLOMEI† + vitamin E§</td>
<td>1540 ± 310†</td>
<td>15.3</td>
<td></td>
<td>39.4</td>
</tr>
<tr>
<td>5% CLOMEI† + IONOX 330*</td>
<td>1540 ± 650‡</td>
<td>15.4</td>
<td></td>
<td>44.0</td>
</tr>
<tr>
<td>5% Lard</td>
<td>1900 ± 370</td>
<td>14.4</td>
<td></td>
<td>37.9</td>
</tr>
<tr>
<td>5% Lard + vitamin E§</td>
<td>2150 ± 530</td>
<td>13.4</td>
<td></td>
<td>33.3</td>
</tr>
</tbody>
</table>

*2,4,6-Tri-(3',5'-di-tert.-butyl-4'-hydroxybenzyl)mesitylene (Shell Ltd), 0.02% of diet.
† Polyunsaturated fatty acids.
‡ Methyl oleate.
§ d-α-tocopheryl acetate, 120 ppm.
I1 Cod-liver oil methyl esters.
 Results for CLOME, considered together, were lower than the remainder (P < 0.01).

Crowne & Hathaway (1965) to be completely non-absorbed. Any effect of this antioxidant would then not be confused with effects in vivo. Seven groups of about sixteen rats were depleted of vitamins A and E as described in Expt 7, except that the maintenance dose of vitamin A was increased to 30 i.u./rat per week. At 8 weeks of age, when one rat was found to have only 16 i.u. vitamin A in its liver, the remaining rats were each given 9550 i.u. vitamin A by mouth and then, the next day, the basal diet with the supplements described in Table 5. The rats were killed when 18 weeks old and their livers were combined in sets of three or four for analysis. The results are given in Table 5. Three different batches of rats were used to compose the groups of sixteen and a significant difference between the batches regardless of diet was allowed for in the evaluation of the results. No significant effect of vitamin E on vitamin A was found in any of the groups, nor was there any effect of IONOX 330 in the rats given CLOME. However, the vitamin A values for all the groups given
CLOME, considered together, were significantly lower than for the other four groups, given either OLME or lard, with or without vitamin E. This effect was not observed in Expts 6 and 7. The effect of CLOME in this experiment cannot be attributed, in any event, to an acceleration of lipid peroxidation either in the gut or in vivo, as it was not affected by the presence of either vitamin E or IONOX 330. The results shown for PUFA in liver and adipose tissue demonstrate the accumulation of polyunsaturated lipid in the tissues of the rats given CLOME. Vitamin E did not influence this.

The effect of vitamin A on the metabolism of α-tocopherol

The antagonistic relationship between vitamins A and E in vitro, which involves vitamin E as an antioxidant, signifies conversely that vitamin A acts as a peroxidative substrate. Thus, if the biological antioxidant hypothesis is valid for conditions in vivo, vitamin A must act as a stress on the vitamin E-deficient animal by destroying, through co-oxidation, residual amounts of vitamin E. The following two experiments examined the effect of vitamin A on the metabolism of small amounts of radioactive α-tocopherol in the chick and the rat.

Expt 9. Twenty-four 1-day-old chicks were given for 21 days the vitamin E-deficient diet containing 18% casein described by Diplock, Bunyan et al. (1967), but modified to contain 4% lard as the fat. The chicks were then given 101.6 μg (11755 dps) [14C]α-tocopherol, in 0.2 ml 0.9% NaCl solution containing Tween 80, by injection into the brachial vein. They were allocated to two groups, so that each group contained four lots of three chicks, and so that each lot in group 1 was matched in weight with a corresponding lot in group 2. The birds in group 1 continued to receive the basal diet and those in group 2 were given the diet with the addition of 0.25% vitamin A acetate. Following Pudelkiewicz, Webster, Olson & Matterson (1964), we opened the eyelids of the birds in group 2 several times daily for the first few days to prevent blindness through incrustation. In agreement with these workers, no other toxic signs were observed. After 11 days the birds were killed and their carcasses (without the alimentary canal) and livers were analysed for [14C]α-tocopherol and vitamin A, the tissues from each lot of three being combined.

Table 6 gives the results. The large vitamin A dosage caused some weight depression in group 2 birds and large amounts of the vitamin were found in the carcasses and livers of this group. There was no evidence of destruction of tocopherol by vitamin A. In fact, the livers of the vitamin A-supplemented birds showed a significant increase in the recovery of [14C]α-tocopherol, compared to controls.

Expt 10. Twenty-seven weanling rats of both sexes were given the vitamin E-deficient diet A10 Y3 from which the vitamin A was omitted from 28 days of age until they were 12 weeks old. During this period the rats received a maintenance dose of 15 i.u. vitamin A palmitate per week. The rats were then marginally deficient in vitamin A and some of them had stopped growing. Each rat was then given a single oral dose of 103.7 μg (12434 dps) [14C]α-tocopherol. After 48 h, the rats were allocated to three groups. Those in group 1 were given a single oral dose of 30 i.u. vitamin A palmitate; those in group 2 a single dose of 10000 i.u.; and those in group 3 received 10000 i.u.
Table 6. Expt. 9. Effect of large amounts of vitamin A on the metabolism of \( \alpha \)-tocopherol in the vitamin E-deficient chick

(Warren cockerels, given a vitamin E-deficient diet for 21 days, were each given 1016 \( \mu \)g (11 755 dps) \( [\text{\textsuperscript{14}}C] \alpha \)-tocopherol intravenously. Group 2 birds were then given the diet with the addition of 0.25% vitamin A acetate, group 1 acting as controls. Each group contained four lots of three birds, each lot matched for weight with a corresponding lot in the other group. The combined tissues of each lot were analysed after 11 days (four analyses per group). Results are given as means with standard deviations)

<table>
<thead>
<tr>
<th>Group</th>
<th>Vitamin A treatment</th>
<th>Initial chick wt (g)</th>
<th>Wt gain (g)</th>
<th>Liver wt (g)</th>
<th>Vitamin A</th>
<th>( [\text{\textsuperscript{14}}C] \alpha )-Tocopherol</th>
<th>( [\text{\textsuperscript{14}}C] )Metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Vitamin A</td>
<td>Carcass* (total i.u.)</td>
<td>Liver (total i.u.)</td>
</tr>
<tr>
<td>1</td>
<td>25 i.u./g diet</td>
<td>167 ± 24</td>
<td>112 ± 21</td>
<td>1122 ± 1.06</td>
<td>844 ± 127</td>
<td>5250 ± 1127</td>
<td>3717 ± 167</td>
</tr>
</tbody>
</table>
| 2     | 0.25% in diet       | 166 ± 23            | 63 ± 31     | 1007 ± 1.37 | 18970 ± 528| 6875 ± 954                 | 3986 ± 237                   | 208 ± 23†         | 2016 ± 407         | 166 ± 25         | * Without alimentary tract. † Significantly higher than value for group 1, \( P < 0.01 \).

Table 7. Expt. 10. Effect of large amounts of vitamin A on the metabolism of \( \alpha \)-tocopherol in rats made deficient in vitamins A and E

(Each group contained nine rats, 12 weeks of age, deficient in both vitamins. Each rat was given a single dose of 101.7 \( \mu \)g (12 434 dps) \( [\text{\textsuperscript{14}}C] \alpha \)-tocopherol and, 48 h later, the vitamin A as shown. The rats were killed on the 13th day of test and their livers and carcasses were analysed in lots of three (each of one male and two females). There were three such analyses per group. Results are given as means with standard deviations)

<table>
<thead>
<tr>
<th>Group</th>
<th>Vitamin A dose (i.u.)</th>
<th>Initial rat wt (g)</th>
<th>Wt gain (g)</th>
<th>Liver wt (g)</th>
<th>Vitamin A</th>
<th>( [\text{\textsuperscript{14}}C] \alpha )-Tocopherol</th>
<th>( [\text{\textsuperscript{14}}C] )Metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Vitamin A</td>
<td>Carcass* (total i.u.)</td>
<td>Liver (total i.u.)</td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>115.9 ± 28.8</td>
<td>-8.1 ± 6.4</td>
<td>5.53 ± 0.78</td>
<td>Not detected</td>
<td>Not detected</td>
<td>2619 ± 106</td>
</tr>
<tr>
<td>2</td>
<td>10 000</td>
<td>114.2 ± 22.0</td>
<td>24.8 ± 9.9</td>
<td>6.59 ± 0.96</td>
<td>Not detected</td>
<td>Not detected†</td>
<td>4103 ± 2237</td>
</tr>
</tbody>
</table>
| 3     | 100 000†            | 109.4 ± 34.2        | 22.5 ± 10.9 | 6.69 ± 0.06 | 1944 ± 51   | 22438 ± 2115    | 3033 ± 108         | 176 ± 29          | 1232 ± 139        | 80 ± 11          | * Without alimentary tract. † See p. 856. ‡ 10 000 i.u. given daily for 11 days.
daily for 11 days. The rats were killed 13 days after the tocopherol dose was given and their livers and carcasses (in three lots, each of one male and two females, per group) were analysed for [14C]α-tocopherol and vitamin A. The results are shown in Table 7. During the 13 days of test many rats in group 1 continued to lose weight and some developed xerophthalmia. Vitamin A was not detected in the carcasses or livers of group 1 animals. Group 2 rats contained about half the administered dose in their livers, but vitamin A was not found in the portion of the carcass extract examined (this indicates only that there was a low concentration in the rest of the tissues, below the level of sensitivity in the particular test, due to the high dilution necessary to measure vitamin A satisfactorily in non-saponifiable extracts of total rat carcass). In group 3, large amounts of vitamin A were found in liver and carcass. There was no evidence that the massive doses of vitamin A had caused any destruction of tocopherol.

Table 8. Expt 11. Onset and incidence of encephalomalacia in vitamin E-deficient chicks given large doses of vitamin A

<table>
<thead>
<tr>
<th>Vitamin A dosage</th>
<th>No. in group</th>
<th>Day when encephalomalacia developed</th>
<th>Survivors at 38 days</th>
<th>Vitamin A in liver* (i.u./g.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 000 i.u.† over 17 days</td>
<td>9</td>
<td>21, 26, 27, 28, 35</td>
<td>1</td>
<td>1265</td>
</tr>
<tr>
<td>10 i.u./g. of diet</td>
<td>9</td>
<td>18, 24, 28, 29, 30, 31</td>
<td>1</td>
<td>480</td>
</tr>
</tbody>
</table>

* Measured in survivors.
† 10 000 i.u. at 10 and 21 days and 20 000 i.u. at 27 days.

A study of the 'pro-oxidative' effect of vitamin A on vitamin E by a biological method

Expt 11. Encephalomalacia in the vitamin E-deficient chick appears to be specifically related to the presence of linoleic acid in the diet. The disease is readily prevented by small amounts of α-tocopherol, and Horwitt, Harvey, Century & Witting (1961) have shown that there is a graded competitive response between linoleic acid and α-tocopherol in the induction of the disease. Diplock, Bunyan et al. (1967) showed that, with chicks given a diet containing about 2% linoleic acid, 1 mg D-α-tocopherol given intraperitoneally produced an α-tocopherol concentration in the cerebellum of about $2 \times 10^{-7}$ g/g lipid and, as a result, delayed the onset of disease by 3.5 days. The onset of encephalomalacia can therefore be used as a sensitive biological index of tocopherol availability in vivo. If the addition of a stress agent decreases this concentration and the over-all nutritional situation is otherwise marginal for disease production, then the stress agent might be expected to precipitate the disease or accelerate its onset.

One-day-old chicks were given for 10 days the vitamin E-deficient diet containing 18% casein (Diplock, Bunyan et al. 1967), but containing 10% OLME as the lipid component. Two groups of nine birds were, from 10 days, given the diet modified by replacing the 10% OLME with 8% glucose and 2% MOME. This diet contained about 1% linoleic acid, which is just sufficient to produce encephalomalacia (Horwitt
One group was given orally 10 000 i.u. vitamin A palmitate on the 10th and 21st days and 20 000 i.u. on the 27th day of test. The second group received only the vitamin A present in the diet. The incidence of encephalomalacia was recorded for each group and the experiment was terminated at 38 days. The results are given in Table 8 and show that the vitamin A stress did not accelerate the onset of disease. There cannot, therefore, have been any significant effect of vitamin A on the vitamin E content of the cerebellum.

**DISCUSSION**

The problem of the relationship between vitamins A and E has received considerable study since Moore (1940) first drew attention to it. There are a number of facets to the problem that are of nutritional importance. These include the dependence of the growth biological assay response for vitamin A on dietary vitamin E, the stability of vitamin A in animal diets, which is also related to their vitamin E content; and the development of vitamin E deficiency in animals given excessive amounts of fish-liver oils containing vitamin A. We are concerned here, however, with the problem of whether the relationship between the two vitamins in vivo can be regarded as one involving a pro-oxidative stress and a biological anti-oxidant. The arguments and methods we have used follow closely those described in earlier papers in this series. Any ‘anti-vitamin E’ stress may be considered to act in two ways: first, by destroying vitamin E in the diet or in the intestinal tract, and, secondly, as the result of an antagonistic relationship in vivo. Typical instances of the first type of stress are the actions of peroxidized fats or pro-oxidative minerals such as iron salts in the diet. Examples of the second type of stress are iron overloading (Golberg & Smith, 1958), Se deficiency (Schwarz, 1962, 1965) and silver toxicity (Diplock, Green et al. 1967). The anti-vitamin E effect of PUFA is probably due to both types of stress relationship (Green, Diplock, Bunyan, McHale & Muthy, 1967). None of the stresses we have so far examined has been found to involve oxidative reactions in vivo. It was therefore essential to study the vitamin A–vitamin E relationship within the general orbit of stress but, as in earlier studies of this series, to examine whether the concept of vitamin A as a ‘pro-oxidant’ or as a ‘co-oxidant’ (Tappel, 1962) can be maintained. Vitamin A is readily autoxidized in vitro, and its oxidation rate is dependent on the usual factors: concentration of substrate, the presence of pro-oxidants such as unsaturated fatty acids, and the presence of antioxidants. If reactions in vivo follow those in vitro mechanistically and kinetically, these factors would be expected to play similar roles in biological systems. However, although the vitamin A–vitamin E relationship has been studied by many workers, the experimental designs used in most instances failed to distinguish between effects in the tissues after absorption and interactions in the gastro-intestinal tract before absorption. Moore (1940) gave female rats eight weekly doses of 1000 i.u. vitamin A, then gave them a vitamin E-deficient diet and some of them graded weekly oral doses of from 0.1 to 3.0 mg DL-α-tocopherol. After 6 months, the livers of the rats given at least 1 mg tocopherol weekly contained about three times as much vitamin A as controls. Davies & Moore (1941) gave young female rats halibut-liver oil until they had accumulated
large reserves of vitamin A and then divided them into two groups, one to receive a diet deficient in vitamins A and E and the other that diet with 1 mg dl-α-tocopheryl acetate weekly. They found that the vitamin A reserves of the rats given tocopherol were greater than of controls; a substantial difference was recorded after 28 days and there was a threefold difference after 91 days. In these two experiments no interaction of the two vitamins in the gut took place, as vitamin A was not given during the experimental period and the only factor under study was the depletion rate (total catabolism) of the stored vitamin A. Moore (1940) described three other experiments; in these, vitamin A was given in weekly doses throughout the test and the tocopherol-supplemented rats received weekly doses of tocopherol at the same time. The tocopherol-supplemented livers contained more vitamin A after 32–56 weeks than the deficient controls. However, in these latter experiments of Moore (1940) any effect of vitamin E on vitamin A depletion may have been confounded with (1) long-term effects of vitamin E deficiency on the absorption of vitamin A, and (2) protection of vitamin A in the gut during the weekly administration. Bacharach (1940) found that the vitamin A reserves of female rats given a tocopherol-deficient diet containing vitamin A for up to 14 weeks were the same as those of controls given sufficient tocopherol to prevent sterility; but when the tocopherol supplement was increased about fifteenfold, a significant increase in vitamin A reserves was found. In this experiment also it is not possible to separate any ‘antioxidative’ protective effect of vitamin E in vivo from the other two effects described above.

Subsequent to the initial observations of Moore (1940) and Bacharach (1940) attention was focused on another aspect of the problem, the effect of vitamin E on the utilization of small quantities of vitamin A for growth. Thus, Hickman, Kaley & Harris (1944a, b) found that daily doses of 0·3 mg of a mixed tocopherol concentrate slightly increased the time taken for weanling rats to deplete their stores of vitamin A and reach their weight plateau. In another test they showed that daily doses of 0·15 mg of the tocopherol concentrate slightly increased the survival time, from 46 to 52 days, after a single dose (50 USP units) of vitamin A. In further experiments, marginal growth-promoting amounts of vitamin A (c. 0·5 μg) were given daily to weanling rats already receiving a diet deficient in vitamins A and E, and, under these conditions, Hickman et al. (1944a) found a marked stimulation of growth by tocopherols, which could be almost entirely eliminated (Hickman et al. 1944b) if the two vitamin supplements were given on alternate days. Hickman et al. (1944b) concluded that the sparing action of vitamin E on vitamin A was chiefly due to suppression of oxidation in and near the gastro-intestinal wall; however, the depletion experiments of Hickman et al. (1944a) are not necessarily accounted for by this explanation. Although Popper & Volk (1944) reported that fluorescence microscopy of rat intestine showed no effect of vitamin E in enhancing the absorption of vitamin A, it is difficult to reconcile their finding with the well-established observations of Hickman et al. (1944b) and many other workers who later confirmed the phenomenon of protective action in the gut. Lemley, Brown, Bird & Emmett (1947) made an even more detailed study of the relationship, using growth response as the criterion. When 2·04 i.u. of vitamin A per day were given to weanling rats receiving a diet deficient in vitamins A and E, 0·3 mg
of a tocopherol concentrate per day stimulated growth. In contrast to Hickman et al. (1944b), however, Lemley et al. (1947) found that the synergistic action was maintained when the two vitamins were given on alternate days, and they concluded that there was a sparing effect of vitamin E on vitamin A in the tissues of the animal. Lemley et al. (1947) studied also the effect of vitamin E on the storage of vitamin A in liver, after giving the two vitamins simultaneously. They found no effect over a period of 3 days, irrespective of the level of vitamin A given (63–4000 i.u./day). In long-term experiments, however, lasting from 3 to 6 months, they found a significant effect of vitamin E on vitamin A storage. In these latter experiments, as in those of Bacharach (1940), the in vivo protective effect was confounded with other effects. Sherman (1947) and Miles, Erickson & Mattill (1949) agreed with Hickman et al. (1944a, b) and Lemley et al. (1947) that the growth response of rats to marginal amounts of vitamin A was increased by simultaneous supplementation with α-tocopherol. Hebert & Morgan (1953), however, found that the addition of 0.5 mg α-tocopherol daily to the diet of rats receiving 35–129 i.u. vitamin A daily for 14–28 days produced no effect on storage of vitamin A, a result essentially in agreement with that of Lemley et al. (1947).

The extensive experiments of Dam, Prange & Søndergaard (1952a, b) on the effect of α-tocopherol and other antioxidants on vitamin A storage in the livers of rats and chicks are of special importance. Dam et al. (1952a) showed that, if chicks were given an exudate-producing diet containing 10% cod-liver oil, the addition of 0.01% DL-α-tocopheryl acetate increased vitamin A storage after 4 weeks almost tenfold; however, when the diet contained 10% lard or was fat-free and the vitamin A was given by mouth, vitamin E supplementation had no effect on vitamin A storage. Identical results were found for rats in experiments lasting 14 weeks (Dam et al. 1952b). Dam et al. (1952a) discussed at some length the nature of the relationships they found, considering three alternatives: (1) interaction in the food before ingestion, (2) interaction in the gut, and (3) interaction in the tissues after absorption. They showed that no destruction of vitamin A occurred in the food before ingestion and, by experiment with an artificial alimentary canal under anaerobic conditions, claimed to have ruled out the possibility that vitamin A could be destroyed in the intestinal tract. They concluded therefore that vitamin A must be subject to peroxidative attack in the tissues of the animal and that vitamin E protected against this destruction by its antioxidant action. However, contrary to the assumptions of Dam et al. (1952a), the stomach and small intestine of the rat and chick must be presumed to have an oxygenic environment, since they contain considerable populations of obligative aerobic microorganisms, but no anaerobes (Smith, 1965). The conclusions of Dam et al. (1952a) are open to further objection. The pronounced difference found between their results for dietary cod-liver oil and those for lard they accounted for by a ‘destructive action on vitamin A connected with autoxidation of highly unsaturated fatty acids in vivo.’ If, in the absence of vitamin E, cod-liver oil fatty acids not only induce exudative diathesis but also destroy vitamin A in the tissues of the animal, exerting both effects by a pro-oxidative mechanism, why then does not lard accelerate vitamin A destruction, for it too induces a high incidence of the disease? The findings of Dam et al. (1952a, b) would seem, indeed, to demonstrate not a relationship between the two
vits in the presence of polyunsaturated fatty acids in the gut. Thus, it is known that lipid peroxidation does take place in the intestinal tract and is profoundly influenced by the balance of dietary minerals, lipids and antioxidants. Green, Diplock, Bunyan, McHale & Muthy (1967) have demonstrated destruction of tocopherol in the gut in the presence of polyunsaturated lipid, and Hickman et al. (1944a) found marked enhancement of the growth response of the rat to marginal daily doses of vitamin A, on giving simultaneous doses of a relatively unabsorbed antioxidant such as laurylhydroquinone.

More recently other workers have found that vitamin E, given in the diet with vitamin A, increases storage of the latter. Thus, Roels, Guha, Trout, Vakil & Joseph (1964) found that, with weanling rats given vitamin A at 1.72 mg/kg diet (somewhat more than their daily requirement), the addition of DL-α-tocopherol at 500 ppm led to significantly greater storage of vitamin A than did the addition of the tocopherol at 50 ppm. This finding seems difficult to account for in terms of a difference between the ‘anti-oxidative’ effects of the two vitamin E levels, for even 50 ppm dietary tocopherol is more than adequate for the rat’s needs; Embree (1947) and Moore (1957) suggest that the vitamin E effect on vitamin A is only generally observed when a vitamin E-deficient diet is used as control. Harrill, Minarik & Gifford (1965), using a 10% lard diet, found that DL-α-tocopheryl acetate, given orally at 10 mg/week, after 12 weeks significantly increased vitamin A storage in rats given 70, 400, or 1200 i.u. vitamin A/week, simultaneously with the tocopherol, in weekly doses; the control rats received a vitamin E-deficient diet in this experiment. It should be noted that the diet, the dosages and general conditions used by Harrill et al. (1965) were very similar to those used by Dam et al. (1952b) and for which the latter authors found no effect of vitamin E on vitamin A storage.

In our experiments, we have studied the vitamin A-vitamin E relationship by seeking evidence of peroxidative attack on vitamin A in vivo. In Expts 1–5 we varied the amount of peroxidizable substrate by including different amounts of highly unsaturated lipid in the diets. None of these affected the concentration of vitamin A in liver, regardless of the method of vitamin A administration; nor, when a necrogenic diet (deficient in Se and tocopherol) was used, was the depletion rate of vitamin A from the liver increased by inclusion of cod-liver oil methyl esters in the diet. In Expt 5, the independent effects of linoleic acid stress and Se deficiency on the development of liver necrosis in the rat was also studied, and it was found that the incidence of disease did not affect vitamin A storage. As already cited, Moore (1940) and Davies & Moore (1941) found marked differences in the depletion rate of vitamin A from the livers of rats given vitamin E compared to vitamin E-deficient controls. In Expts 6–8, we studied the long-term effects of dietary lipid (co-oxidizable substrate) and vitamin E (the ‘antioxidant’) on the total catabolism of vitamin A by measuring depletion of liver reserves after a single dose. In general, the higher unsaturated cod-liver oil methyl esters (CLOME) did not accelerate vitamin A destruction and, indeed, they increased the immediate (24 h) retention of vitamin A (Expt 7). In Expt 8, however, the vitamin A reserves of the three groups of rats given CLOME were significantly lower than those given methyl oleate (OLME) or lard. Neither vitamin E nor
the almost unabsorbed antioxidant IONOX 330 reversed this trend, suggesting that the CLOME was not acting as a pro-oxidant. The similarity between the results for rats given CLOME with vitamin E and CLOME with IONOX 330 provides further evidence against an antioxidant effect of vitamin E in vivo. This depressant effect of CLOME was not found in Expts 6 and 7 and may have been made more apparent in Expt 8 by the presence of the higher vitamin A levels for the rats given OLME with vitamin E and lard with vitamin E. A sparing effect of vitamin E was only shown significantly in part of Expt 7 at 4½ weeks after the vitamin dose, but was not found when similar rats were examined after 12½ weeks. Consideration of all the nine vitamin A values, in Expts 6–8, for rats given vitamin E showed that they were higher than for controls \( P < 0.05 \), but only in the single-tailed \( t \) test. In fact, two of the nine results were lower than controls. We were not able to account for the differences between our results and those of Moore (1940) and Davies & Moore (1941). Their strain of rat was different from ours and they used animals with initial vitamin A reserves about ten times those of our rats. More recently the reasons for the differences between our results and those of Moore (1940) have been established (Cawthorne, Bunyan, Diplock, Murrell & Green, 1968).

In Expts 9–11 we studied the converse relationship; that is, the effect of a putative ‘peroxidative’ stress due to vitamin A on the metabolism of \( \alpha \)-tocopherol. Previous workers have demonstrated that excessive amounts of dietary vitamin A can lead to a depletion in tissue vitamin E, detectable either by analysis (Edwin, Bunyan, Green & Diplock, 1962; Pudelkiewicz, Webster & Matterson, 1964) or by clinical signs of vitamin E deficiency (Irving, 1958). Brubacher, Schärer, Studer & Wiss (1964–5) found that the recovery of \( [14C] \alpha \)-tocopherol from the livers of rats given a single dose was depressed if that dose was accompanied by a simultaneous dose of vitamin A. In all these experiments, however, the two vitamins were allowed to interact in the gastro-intestinal tract. Furthermore, giving excessively large amounts of vitamin A in the diet with small amounts of vitamin E may depress the absorption of the latter (cf. Pudelkiewicz, Olson, Matterson & Suden, 1964). In two experiments in which we gave large doses of vitamin A to vitamin E-deficient chicks and rats previously given a small dose of \( [14C] \alpha \)-tocopherol, we found no evidence of any increased destruction of the tocopherol due to vitamin A. Indeed, the trend was always the reverse, somewhat more tocopherol being recovered from animals treated with vitamin A; and in Expt 9 a significantly increased recovery of tocopherol was found in the vitamin A-treated livers. An attempt (Expt 11) to demonstrate a destructive effect on tocopherol due to vitamin A by studying the effect of the latter on the rate of onset of encephalomalacia in vitamin E-deficient chicks failed (cf. Diplock, Bunyan et al. 1967, who found that the rate of onset of disease was influenced by the cerebellar concentration of \( \alpha \)-tocopherol). The results of this experiment do not confirm the suggestion of Prohászka (1966) that excessive dosage with vitamin A can induce encephalomalacia. Prohászka’s (1966) conditions were different from ours, however, and his diets were of a commercial type and contained 10% oxidized cottonseed oil; it should be noted, though, that in his chicks treated with vitamin A the livers contained the same concentration of vitamin E as those of untreated controls.
To summarize so far, it would seem essential to distinguish possible separate effects of vitamin E on different aspects of vitamin A metabolism: uptake, which may depend on an effect of vitamin E on the absorption process; retention, which may depend on an effect of vitamin E on the storage mechanism of the liver; and utilization, which may be effected in more specialized tissues. It is unlikely that an effect of vitamin E on vitamin A utilization at the cellular level can be demonstrated in experiments designed to study effects on vitamin A uptake and storage. Factors such as dietary composition and fat content, method of vitamin dosage, interactions in the gut, and the time intervals involved would all seem to be important in accounting for apparent inconsistencies between different workers’ findings. It must be noted, furthermore, that vitamin E may have significant effects, irrespective of vitamin A, on the general lipid metabolism of the liver, which may itself influence vitamin A storage (Harrill et al. 1965). The most marked effects on liver storage are observed when the diet contains highly unsaturated lipid, especially cod-liver oil (Dam et al. 1952a, b), but this does not seem to be a necessary criterion, as the diet of Harrill et al. (1965) contained lard (10%) as the only fat. Depletion experiments are of two kinds. In the first, the rate of decrease of an initially high liver reserve is studied with and without vitamin E. Here, in three large experiments, we were unable to confirm the apparently clear results of Moore (1940) and Davies & Moore (1941). In the second type of depletion study, weanling rats with initially low reserves of vitamin A are allowed to deplete their stores until growth ceases and the influence of vitamin E is studied; experiments of this kind probably deal essentially with the function of vitamin A in the same terms as experiments on the utilization of small quantities for growth. There is some evidence, as we have seen, that vitamin E-supplemented rats utilize vitamin A better than deficient rats, although the reasons for this are still not clear. The experiments of Hickman et al. (1944a, b) have the most satisfactory design and their experimental period was probably short enough for there to have been no secondary pathological effects of vitamin E deficiency. Of all the various experiments carried out on the effects of vitamin E on vitamin A utilization, only those of Hickman et al. (1944a, b) and Lemley et al. (1947) included some designed to dissociate possible interactions in the gut from those in the tissues of the animal after absorption; and they produced diametrically opposite results. It may be important, however, that Lemley et al. (1947) gave considerably more than the marginal amounts of vitamin A used by Hickman et al. (1944b), and the problem of recirculation of both vitamins into the vascular system of the intestinal wall, as suggested by the latter authors, may under certain conditions be critical to the manifestation of the effect. α-Tocopherol is known to recirculate from the vascular system into the gut itself (Green, Diplock, Bunyan, McHale & Muthy, 1967); but in an experiment (unpublished) in which we gave [14C]retinyl acetate (161 μg) intravenously to rats simultaneously given large amounts of non-radioactive retinyl palmitate by mouth, less than 0.05% of the intravenous dose was recovered from the entire gastro-intestinal tract and faeces during the subsequent 3 days. If vitamin E does affect the utilization of vitamin A, it is in any event unnecessary to relate this to antioxidant effects. Similar effects on vitamin A utilization can be produced by factors other than vitamin E. McCarthy & Cerecedo (1952) found that, in the mouse, the rate of vitamin A depletion...
was markedly accelerated not only by the withdrawal of vitamin E from the diet but by the withdrawal of fat. Such an effect would, at first sight, appear impossible to explain by peroxidation and antioxidant relationships in vivo. The relationship between vitamin A and dietary antibiotics, in its inconsistencies at least, is virtually a facsimile of the vitamin A–vitamin E relationship. Thus, Burgess, Gluck, Brisson & Laughland (1951) found that dietary penicillin increased growth and liver storage of vitamin A in chicks given a commercial diet. Hartsook, Batchelor & Johnson (1953) found that aureomycin did not increase the utilization of vitamin A in the male rat, as judged by liver storage, growth or survival time: the unpurified diet contained 5% cottonseed oil, and the vitamin A was given in daily doses, with the addition of \(\alpha\)-tocopherol. Murray & Campbell (1955a, b), however, using a laboratory diet with 5% maize oil, showed that aureomycin increased the utilization of vitamin A in the ovariectomized rat, as measured by the vaginal smear assay; did not increase liver storage of vitamin A given in the diet or by oral dosing; did not influence the rate of depletion of vitamin A reserves in kidney or liver; increased the depletion time, weight at depletion and survival time of ovariectomized vitamin A-deficient rats; and increased the growth rate of rats receiving only 2 i.u. vitamin A/day, but not of rats given adequate doses of vitamin A. Reber, Morrill, Norton & Rhoades (1956), in contrast to Murray & Campbell (1955b), found that aureomycin and \(\alpha\)-tocopherol could independently increase liver storage of vitamin A; but the diet they used contained 40% fat, of which 2% was cod-liver oil. It is of interest that Murray & Campbell (1955a) found no effect of \(\alpha\)-tocopherol on the utilization of vitamin A in the ovariectomized rat.

In conclusion, it seems that any relationship that may exist in vivo between vitamins A and E is not concerned with an effect of the latter in preventing oxidation of the former. There is clearly still a need for more definitive experiments on the mode of action of vitamin E in the vitamin A growth test. There is considerable recent evidence that the biochemical relationships of the fat-soluble vitamins may be subtle and complex. Thus, Seward, Vaughan & Hove (1964), considering the action of vitamins A and E on respiration of liver homogenates, conclude that they may both effect changes in membrane permeability; Roels, Trout & Guha (1965), studying lysosomal stability, reached a similar conclusion; Guha & Roels (1965), studying the effects of \(\alpha\)-tocopherol on arylnitrates in vitamin A-deficient rat liver, believe that ‘the action of vitamins A and E...is considerably more complex than mere peroxidation and may be due to an activation effect of an enzyme capable of attacking membrane components, or to a direct or indirect effect of the vitamins on the membrane structure itself’. Brown, Button & Smith (1963) have suggested that the fat-soluble vitamins may all act through at least one common function, perhaps related to changes in the sulphation of mucopolysaccharides associated with cellular lipoproteins. Olson (1964) has postulated that the fat-soluble vitamins may affect some stage in the transmission of genetic information in the cell.

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