On the relationship between vitamin A and vitamin E in the rat

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1. The effect of vitamin E on the metabolism, utilization and storage of vitamin A has been studied in the rat.

2. Male weanling rats were given a vitamin A-deficient, vitamin E-deficient diet until growth had ceased for 3 days, and each rat was then given 50 i.u. vitamin A palmitate. The rats were divided into four groups and given the diet with the addition of 10 % methyl oleate or 10 % cod-liver oil methyl esters, or either of these diets supplemented with 100 ppm D-α-tocopheryl acetate. There was no increase in maximum weight-gain response in the two groups given vitamin E. There was a significantly lower weight-gain response in the groups given cod-liver oil methyl esters. This effect was not influenced by the presence of vitamin E in the diet.

3. Weanling rats of both sexes were made deficient in vitamins A and E and then divided into two groups. One group received, every other day, 1·75 i.u. vitamin A palmitate and 0·6 mg D-α-tocopherol given together; the second group received the two vitamins, in the same amounts, on alternate days. After 28 days there was no difference in the growth of the two groups of rats, irrespective of sex.

4. Vitamin A-depleted, vitamin E-deficient rats were given 17·51 μg [^{14}C-carbinol]retinyl acetate and then a vitamin A-deficient, vitamin E-deficient diet or that diet supplemented with 100 ppm D-α-tocopheryl acetate. After 6 days, the total remaining [^{14}C]retinol and its lipid-soluble metabolites were measured in the carcasses of the rats. Vitamin E administration did not affect the metabolism of the vitamin A dose or its effect on growth.

5. Vitamin E-deficient rats were given vitamin A until their liver reserves exceeded 30 000 i.u. and were then divided into two groups. One group received a diet deficient in vitamins A and E and the other received, in addition to this diet, a weekly oral supplement of 1 mg D-α-tocopheryl acetate. The vitamin E supplement significantly decreased the rate of vitamin A depletion from the liver during the next 6 weeks. This effect, which was not found to occur when the initial liver reserves were only 3000 i.u., suggests a role for vitamin E in connexion with the capacity of the liver to bind vitamin A.

6. The relationship between vitamin A and vitamin E in vivo cannot, in the light of these results, be regarded as that between an antioxidant and a peroxidizable substrate.

We have previously described some studies on the relationships between vitamin E, vitamin A and dietary lipid in the chick and the rat and have discussed the problem of the vitamin A–vitamin E relationship in terms of the biological antioxidant hypothesis (Green, Muthy, Diplock, Bunyan, Cawthorne & Murrell, 1967). There is now much evidence that the concept that vitamin E deficiency diseases are caused by the 'proliferation' of lipid peroxidation in vivo is untenable (Green, Diplock, Bunyan, McHale & Muthy, 1967; Bunyan, Murrell, Green & Diplock, 1967) and any role for vitamin E as a physiological antioxidant would now seem difficult to support. If, therefore, a true 'sparing' effect of vitamin E on vitamin A exists in vivo, the cause must lie elsewhere than in a simple 'pro-oxidant–antioxidant' relationship.

In our previous publication (Green, Muthy *et al.* 1967) it was suggested that most of the experiments of earlier workers on the vitamin A–vitamin E relationship con-

founded a possible sparing effect in the tissues with the more certain interaction of the two vitamins in the gastro-intestinal tract. Attention was drawn to the contradictory nature of many of the reports, which, we believe, may be explained by differences in the dietary conditions used by various workers, with particular respect to the presence of natural antioxidant substances other than α-tocopherol. Two problems, however, seemed to demand further study. One of these was whether, in the rat, vitamin E exerted a true physiological sparing effect on the utilization of small amounts of vitamin A for growth, at levels below those necessary to promote storage in the liver; and we drew specific attention to the contradictory findings on this subject, of Hickman, Kaley & Harris (1944a, b) and Lemley, Brown, Bird & Emmett (1947). The second problem was as follows: we found that vitamin E did not affect the depletion rate of vitamin A from rat liver, under conditions in which interaction of the two vitamins in the gastro-intestinal tract was avoided; but Moore (1940) and Davies & Moore (1941) found a very large effect of vitamin E on the depletion rate of vitamin A, under similar conditions. We have carried out some further experiments on these matters.

EXPERIMENTAL AND RESULTS

Animals and diets. Norwegian hooded rats were used except in Expt 4 where albino rats were also used. The rats were given the vitamin E-deficient diet A 10 Y 3 (Bunyan, McHale & Green, 1963) until the start of each experiment. The rats were allocated to experimental groups at random with litter-mate control. In the experiment with [^{14}C]retinol, the rats were housed individually in tubular cages to prevent coprophagy (Green, Diplock *et al.* 1967).

Materials. Methyl oleate (OLME) and cod-liver oil methyl esters (CLOME) were prepared free from vitamin E as described by Green, Diplock *et al.* (1967). [^{14}C-carbinol]retinyl acetate was obtained from the Radiochemical Centre, Amersham.

Analyses. Rat carcasses (without alimentary tracts) were frozen at $-20°$ immediately after death until analysed. Livers were either analysed immediately after removal from the animals or were kept at $-20°$ until required. Vitamin A in liver was measured by the method of Ames, Risley & Harris (1954). For the measurement of [^{14}C]retinol, the carcasses of three rats were combined and chopped into small pieces while still frozen. They were saponified by the method of Mervyn & Morton (1959) after preliminary comminution for 1 min with the requisite amounts of ethanol and pyrogallol in an Atomix blender. Retinyl palmitate (10 mg) and D-α-tocopherol (10 mg) were added before comminution to act as carrier for the radioactive vitamin A and as an antioxidant respectively. One two-hundredth of the non-saponifiable extract was taken and the retinol was separated as described in general by Diplock, Green & Bunyan (1963). Sterols were removed by treatment with digitonin and the extracts were chromatographed on Whatman no. 4 paper, impregnated first with zinc carbonate and sodium fluorescein and then with liquid paraffin. The mobile phase was 65 % (v/v) aqueous ethanol and time of running was 6 h. Retinol migrated with R_f 0·8 and was identified by co-chromatography with an authentic marker. It was eluted from the paper and its radioactivity measured, as described by Green, Diplock *et al.* (1967) for

the measurement of $[^{14}C]\alpha$-tocopherol. Where strong quenching was encountered, the correction by the channels ratio method was checked by diluting the samples and recounting them.

Effect of vitamin E and polyunsaturated fatty acids on the growth-promoting activity of vitamin A

Expt 1. In this experiment, we tried to repeat the experiment of Hickman *et al.* (1944*a*), in which the 'sparing' effect of vitamin E in vivo was methodologically separated from any interaction between the two vitamins in the alimentary tract. Thirty-nine male rats were depleted of vitamin A and made deficient in vitamin E by giving them, from 18 days of age, the vitamin E-deficient diet A 10 Y 3 from which the vitamin A had been removed. When each rat reached its weight plateau (no increase for 3 consecutive days), it was given, on the 4th day, 50 i.u. vitamin A palmitate orally and, on the 6th day, allocated to one of four groups treated as follows: group 1 was given the vitamin A-deficient, vitamin E-deficient diet containing 20 % casein described by Green, Diplock *et al.* (1967), with the addition of 10 % OLME; group 2 the diet with 10 % OLME and 100 ppm D-α-tocopheryl acetate; group 3, the diet with 10 % CLOME; group 4, the diet with 10 % CLOME and 100 ppm D-α-tocopheryl acetate. The lipids were all added in replacement of sugar. Each rat was weighed daily until it again reached its weight plateau, and its maximum weight gain was recorded. The maximum was on average reached in all four groups after about 14 days. The results are given in Table 1. The maximum growth response was unaffected by the presence of vitamin E, irrespective of the nature of the dietary lipid. However, the growth response was significantly depressed when CLOME replaced OLME in the diet.

Table 1. *Expt* 1. *Maximum growth response of vitamin E-deficient and -supplemented rats to a single dose of vitamin A, and the effect of polyunsaturated fatty acids*

(Male rats, of about 80 g in weight, that had been given a vitamin A-deficient, vitamin E-deficient diet (see above) and had reached their weight plateau were given 50 i.u. vitamin A palmitate and then one of the four supplemented diets. Maximum weight gain was recorded for the following 3 weeks. Results are given as means with standard deviations)

Group	Dietary supplement	No. of rats	Maximum wt gain (g)
1	OLME*	11	30·0± 7·8
2	OLME*+vitamin E†	10	28·2±10·6
3	CLOME‡	7	18·0±10·8§
4	CLOME‡+vitamin E†	11	20·8±16·0§

* Methyl oleate at 10 % in the diet.
† D-α-Tocopheryl acetate at 100 ppm.
‡ Cod-liver oil methyl esters at 10 % in the diet.
§ The gains with CLOME were significantly less than those with OLME, irrespective of the vitamin E supplementation ($P < 0.05$).

Expt 2. We have previously drawn attention (Green, Muthy *et al.* 1967) to the important experiments of Hickman *et al.* (1944*b*) and Lemley *et al.* (1947), who both tried to decide whether the sparing effect of vitamin E on vitamin A is due to an effect of vitamin E in the intestinal tract or in the tissues after absorption. Their results were

contradictory, and the present experiment was an attempt to confirm one or the other. Sixty-seven rats, including both sexes, were made deficient in vitamins A and E, as described in Expt 1. When each rat reached its weight plateau (no growth increase for 3 consecutive days) it was allotted to one of two groups. Group 1 rats received, every other day, 1·75 i.u. vitamin A palmitate and 0·6 mg D-α-tocopherol together by mouth. Group 2 rats received the same doses, but the vitamin A and vitamin E were given on alternate days. Thus, both groups received the same weekly total intake of 6·1 i.u. vitamin A and 2·1 mg α-tocopherol. Both groups were given the 20 % casein diet, deficient in both vitamins, as used in Expt 1. Their weights were recorded daily for 28 days. Some rats grew steadily during this time, but some increased in weight, then declined and died. Results (Table 2) were assessed by comparing the mean weight gains at 14 days and 28 days. Even allowing for sex differences, no significant difference due to the mode of presentation of the vitamin supplements was found.

Table 2. *Expt 2. Comparison of simultaneous and alternate-day dosing of vitamins A and E to rats made deficient in both vitamins*

(Rats that had been made deficient in vitamins A and E (see p. 135) were allocated to two groups to receive the doses of vitamins A and E, described below. Weights and weight gains are recorded as means with standard deviations and the number of rats is shown in parentheses)

		Wt gain	
Treatment	Wt at start (g)	At 14 days (g)	At 28 days (g)
Every other day, vitamin E* and vitamin A†; alternate days, none:			
Males	83·3 ± 27·1 (13)	14·4 ± 15·8 (10)	35·7 ± 19·9 (7)
Females	89·9 ± 19·1 (19)	16·5 ± 12·5 (17)	30·6 ± 19·6 (16)
Every other day, vitamin A†; alternate days, vitamin E*:			
Males	87·9 ± 30·8 (16)	19·6 ± 13·9 (12)	30·8 ± 15·0 (8)
Females	85·1 ± 20·5 (18)	16·4 ± 15·5 (16)	31·1 ± 18·6 (13)

* A single oral dose of 0·6 mg D-α-tocopherol.
† A single oral dose of 1·75 i.u. vitamin A palmitate.

Effect of vitamin E on the utilization and metabolism of vitamin A

Expt 3. Green, Muthy *et al.* (1967) stressed the need to distinguish a possible effect of vitamin E on vitamin A storage from any effect it may have on the utilization of vitamin A. This experiment was designed to study specifically utilization by giving small doses of radioactive vitamin A to vitamin A-deficient animals whose metabolic needs were such that storage in the liver would be minimal. Forty-three rats, of both sexes, were made deficient in vitamins A and E by giving them, from 18 days of age, the vitamin E-deficient diet A 10 Y 3 from which vitamin A had been removed. At 30 days of age, the rats were given a single oral dose of 30 i.u. vitamin A palmitate in order to extend the time taken to produce vitamin A deficiency and so allow them to become more deficient in vitamin E. The rats began to enter the 'plateau' period of growth cessation at about 9 weeks of age. Twenty-four of the rats were then selected and allocated to eight lots of three, each lot containing one female and two male rats,

so that the total weight of each lot of three was similar. All twenty-four rats chosen had shown no weight gain for the 3 previous consecutive days. Some also showed early signs of xerophthalmia. The rats were then given 17·51 µg (5824 disintegrations per sec (dps)) [¹⁴C-carbinol]retinyl acetate orally in an aqueous emulsion with Tween 80. After 24 h, four of the lots (group 1) were given the vitamin A-deficient, vitamin E-deficient 20 % casein diet described by Green, Diplock *et al.* (1967), containing 5 % lard in replacement of sugar. The other four lots (group 2) received this diet with the addition of D-α-tocopheryl acetate, 100 ppm. The animals were weighed daily and killed after 6 days. The combined carcasses of each lot were analysed for radioactive

Table 3. *Expt 3. Metabolism of [¹⁴C]retinol in vitamin E-deficient and vitamin E-supplemented rats*

(Rats about 9 weeks old and initially deficient in vitamins A and E were divided into four groups. Groups 1 and 2 each consisted of twelve rats, mean weight 92 g, that were allocated to four lots of three (see p. 136). These twenty-four rats were each given 17·51 µg (5824 dps) [¹⁴C-carbinol]retinyl acetate, by mouth. The next day, group 1 was given the diet deficient in vitamins A and E (see above), and group 2 received that diet supplemented with 100 ppm D-α-tocopheryl acetate. These rats were killed after 6 days and their carcasses (without alimentary tracts) were analysed for total residual [¹⁴C]retinol and its metabolites. Four analyses (each on the combined tissues of three rats) were carried out for each group. Groups 3 and 4, consisting of nine or ten rats of mean weight 72 g, were given an oral dose of 50 i.u. vitamin A palmitate (equivalent to the radioactive dose) and then, the next day, the diets described, respectively, for groups 1 and 2. The weight gains of groups 3 and 4 were measured for 16 days. Results are given, where necessary, as means with standard deviations)

Group		Wt gain during 5 days* (g)	[¹⁴C]retinol† dps	[¹⁴C]retinol† % of dose	[¹⁴C]metabolites† dps	[¹⁴C]metabolites† % of dose
Group 1 (E-deficient):	Males	18·9±4·2 (7)	519±88 (4)	9	229±44 (4)	4
	Females	9·5±3·1 (4)				
Group 2 (E-supplemented):	Males	16·1±5·2 (8)	576±71 (4)	10	196±27 (4)	3·5
	Females	5·3±4·6 (4)				
		Maximum wt gain* during 16 days (g)				
Group 3 (E-deficient):	Males	15·0; 2·0 (2)	—	—	—	—
	Females	22·1±6·5 (8)	—	—	—	—
Group 4 (E-supplemented):	Males	52·0; 30·0 (2)	—	—	—	—
	Females	16·1±6·7 (7)	—	—	—	—

dps, disintegrations/sec.
* No. of rats shown in parentheses.
† No. of analyses shown in parentheses.

vitamin A. Table 3 gives the results. There was no difference in the weight gains over 5 days between the vitamin E-deficient and vitamin E-supplemented animals. Neither was there any difference, in the total recovery of radioactive vitamin A from the two groups, in total lipid-soluble [¹⁴C]metabolites. About 90 % of the administered 17·51 µg (*c.* 50 i.u.) had been metabolized by the rats during 6 days.

The remaining nineteen rats, on reaching their weight plateaus, were given 50 i.u.

vitamin A palmitate and divided into two groups (3 and 4) to receive, 24 h later, the vitamin E-deficient or supplemented diet, and their weight gains were studied for the following 16 days. As shown in Table 3, vitamin E did not affect their growth, and this was in agreement with the results found for the rats given the radioactive vitamin A.

Effect of vitamin E on storage of vitamin A in liver

Expt 4. If vitamin E has a role in vivo in increasing the ability of the liver to store vitamin A or in decreasing the rate at which the store is depleted, such effects can only be disclosed in experiments in which interaction of the two vitamins before absorption is eliminated. Of the experiments reviewed by Green, Muthy *et al.* (1967), only those of Moore (1940) and Davies & Moore (1941) were satisfactory in this respect. They found large differences in the depletion rates of initially high liver stores of vitamin A, depending on whether the rats were given diets with or without vitamin E. However, Green, Muthy *et al.* (1967) did not find this, even when diets similar to those described by Moore (1940) and Davies & Moore (1941) were used. In considering the contradiction, two differences between the experiments emerged. Our rats were of a different strain and different size from those of Moore (1940); and, whereas in our experiments the initial total liver stores were never higher than about 3000 i.u., the initial levels used by Moore (1940) and Davies & Moore (1941) were about ten times these. In the present experiment the depletion of such large vitamin A stores from both strains of rat was studied.

Table 4. *Expt* 4. *Effect of vitamin E on the depletion of large reserves of vitamin A from albino and hooded rats*

(Albino female rats were obtained from a commercial breeder. Hooded rats were 3-month-old vitamin E-deficient females. All the rats were given vitamin A palmitate in repeated oral doses until tests showed that livers of rats similarly treated contained at least 30000 i.u. vitamin A. They were then given a vitamin A-deficient, vitamin E-deficient diet (see p. 139) and some rats of each strain were given a weekly supplement of vitamin E. After 6 weeks they were killed and their livers (in pairs) analysed for vitamin A. Results are given as means with standard deviations)

Rat strain	Initial wt range (g)	Treatment	No. in group	Vitamin A/liver Initially* (i.u.)	After 6 weeks (i.u.)
Albino	106–146	Deficient diet	10	39300 ± 5000	34000 ± 4180
		Supplemented†	10	—	44700 ± 5770‡
Hooded	102–156	Deficient diet	10	37300 ± 5500	10300 ± 1410
		Supplemented†	8	—	27550 ± 2190‡

* These values were determined on three rats of each strain treated similarly. They provided an indication of the expected starting levels of vitamin A in the liver.

† These rats were given 1 mg D-α-tocopheryl acetate as a single dose every week.

‡ These values are significantly higher than those of the unsupplemented control groups ($P < 0.001$).

Hooded rats used were 3-month-old females from our vitamin E-deficient colony (102–156 g in weight). Albino females were bought from a commercial breeder and had a weight range of 106–146 g. All the rats were given the vitamin E-deficient diet A 10 Y 3 and repeated daily oral doses of vitamin A palmitate until the liver reserves

of some killed and tested were found to be at least 30000 i.u. vitamin A/liver. Twenty rats of each strain were then taken and divided into groups of ten. The two groups of albino rats were given a diet deficient in vitamins A and E, similar to that described by Moore (1940) and with the percentage composition: casein ('low vitamin content'; Genatosan Ltd) 20, dried brewer's yeast 10, lard 5, salt mixture 3·9, vitamin mixture 0·4 sugar, 40·5, glucose 20·2. The salt mixture was as described by Green, Diplock *et al.* (1967), except that it supplied only 1·17 % $NaH_2PO_4.2H_2O$. The vitamin mixture was as described by Diplock, Green, Bunyan, McHale & Muthy (1967) and contained no vitamin A. One group received each week a single oral dose of 1 mg D-α-tocopheryl acetate per rat. The hooded rats were given the 20 % casein diet, deficient in vitamins A and E, similar to that used in our previous experiments (Green, Diplock *et al.* 1967), with the addition of 5 % lard in replacement of sugar. One group received the weekly dose of vitamin E as above.

After 6 weeks on these diets, the rats were killed and their livers analysed in pairs for vitamin A. Table 4 gives the results. Vitamin E significantly decreased the rate of depletion of the vitamin A reserves in both strains of rat, thus confirming the results of Moore (1940) and Davies & Moore (1941). The effect was less in the albino rats than in the hooded rats. This may have been due to the fact that the albinos had previously been given an adequate diet and so their vitamin E depletion time was not as long as that of the hooded rats.

DISCUSSION

The concepts of 'lipid peroxidation' in vivo and the role of α-tocopherol as a biological antioxidant cannot now be regarded as tenable; for, as we have shown previously (Green, Diplock *et al.* 1967; Bunyan *et al.* 1967) the essential prerequisites on which these concepts depend have not been satisfied. If, therefore, a relationship between vitamins A and E exists in vivo it must be assigned to causes other than that between a 'pro-oxidizable' or 'co-oxidizable' substrate and an antioxidant. Green, Muthy *et al.* (1967) considered that the nature of the relationship in vivo could only be studied satisfactorily in experiments the design of which eliminated the possibility of the two substances interacting before absorption. It was considered then that most of the effects of vitamin E on vitamin A recorded in the literature were best accounted for by the protective action of α-tocopherol on vitamin A in the gut before absorption. However two series of experiments remained of critical importance in attempts to understand the true nature of the relationship. The first were those of Moore (1940) and Davies & Moore (1941) who demonstrated, under experimental conditions precluding interaction before absorption, that vitamin E markedly decreased the depletion rate of vitamin A stored in the liver; but Green, Muthy *et al.* (1967), using somewhat different experimental conditions, were unable to confirm those findings. The second important experiments that seemed to warrant further study were those of Hickman *et al.* (1944*a, b*) and Lemley *et al.* (1947), both of whom studied, also apparently under conditions in which gut interaction was avoided, the effect of vitamin E on the utilization of vitamin A for growth but who produced contradictory results. We have attempted to study further both types of relationship.

Hickman *et al.* (1944*a*) studied the effect of vitamin E on the utilization of vitamin A for growth in two experiments. In one, they measured the time taken, using death as the criterion, for weanling rats given a vitamin A-deficient diet to become completely depleted of vitamin A. They found that when the rats were given vitamin E there was a small but significant effect in increasing survival in males, but not in females. They also found that the tocopherol-supplemented animals gained more weight during the depletion period than controls. In their second experiment, Hickman *et al.* (1944*a*) gave young rats already depleted of vitamin A (presumably the rats had reached the stage of growth cessation, although this was not stated) a single 50 i.u. dose of vitamin A and then studied their maximum growth response, the duration of the response and the survival time, some rats being given daily doses of vitamin E. They found that tocopherol slightly (but not significantly) increased survival time and produced 'a relatively small increase in weight gain' during the experimental depletion period. Examination of their experimental findings, however, shows that, in fact, the maximum weight gain in the animals given vitamin E was considerably less than controls, especially during the first 2 weeks of depletion, and the effect of vitamin E dosage was not to increase maximum growth response but to extend the duration of the growth response. This effect is shown even more clearly in the rats used by Hickman *et al.* (1944*a*) as double negative controls; these rats received no vitamin A and the experimental results demonstrate that supplementation of these rats with vitamin E caused a greater loss in weight during the initial stages of the depletion but an increase in the rats' survival time.

In any event, there is a further difficulty in assessing the results of the experiments of Hickman *et al.* (1944*a*), for vitamin E supplementation may itself affect the growth of weanling rats, even those given adequate vitamin A, providing the diet contains fat. Thus, Century & Horwitt (1960) found vitamin E to increase the growth of rats given diets containing even a relatively saturated fat such as olive oil. The best criterion of vitamin A utilization (in the strict sense of the term; i.e. efficiency of function at the molecular level, rather than perhaps through more indirect physiological mechanisms) may therefore be not survival time but the maximum growth response to a small single dose over a relatively short period of time. Considering, therefore, the results of our Expt 1, no effect of vitamin E on the maximum growth response to a single vitamin A dose was found, and in this respect the results agree with those of Hickman *et al.* (1944*a*). There was, however, a significant effect of polyunsaturated fatty acids (CLOME) in depressing the growth response, but vitamin E did not reverse this. This result provides an interesting example of how a specific toxic effect of cod-liver oil fatty acids (first proposed by Blaxter, 1957) may be erroneously regarded as a 'pro-oxidant' effect (cf. Green, Muthy *et al.* (1967) for another example, in connexion with the effect of cod-liver oil fatty acids on vitamin A storage).

In Expt 2, we studied further the difference of opinion expressed by Hickman *et al.* (1944*b*) and Lemley *et al.* (1947) as to whether the effect of vitamin E on the response to vitamin A was due to a protective effect in the gut or in the tissues. Hickman *et al.* (1944*b*) gave rats 0·4 μg vitamin A every other day and 0·15 mg mixed tocopherols either on the same day or on the alternate day. They found a marked difference in the

total weight gains over 36 days between the two groups. They regarded this result as 'good reason to believe that the effect is exerted chiefly in the intestinal tract', reconciling it with the results of Hickman *et al.* (1944*a*) by suggesting that 'vitamin E... diffusing inwards through the intestinal mucosa, protects the A vitamins even when these are not present in the intestine'. Nevertheless, Lemley *et al.* (1947) repeated the experiment, giving 12·24 i.u. vitamin A and 1·8 mg mixed tocopherols per week to vitamin A-depleted rats, and found that the weight gain in 28 days was the same whether the two vitamins were given on the same day or alternate days; both groups of rats showed a greater weight response than controls given no vitamin E at all. They concluded that the effect of vitamin E on vitamin A could not be confined to the intestinal tract. However, it seemed possible to us that Lemley *et al.* (1947) were unable to repeat the results of Hickman *et al.* (1944*b*) because they had given too much vitamin A for its effect to be limiting. Thus Hickman *et al.* (1944*b*) had already shown that by giving 0·57 μg vitamin A every alternate day, the synergistic effect of vitamin E was markedly reduced. There were also differences in the vitamin A preparations used; Hickman *et al.* (1944*b*) used pure vitamin A acetate or alcohol, and Lemley *et al.* (1947) used a USP reference oil or a commercial vitamin A distillate. The extent of the differences found on alternate-day versus same-day dosage would probably depend on the stabilities of the preparations and the time of day they were administered in relation to the food given. In our Expt 2 we administered the alternate-day doses of vitamin A palmitate in amounts similar to those used by Hickman *et al.* (1944*b*) and gave doses of 0·6 mg D-α-tocopherol either on the same day as the vitamin A or on the alternate day. We found no real difference in the weight gains over 28 days, although individual rats varied widely in their response, and so we could not confirm the contention of Hickman *et al.* (1944*b*) that the effect of vitamin E is confined to a protective action in the intestinal tract. However, further examination of these results in conjunction with all those in the relevant literature have led us to conclude that the problem of interaction between vitamins A and E before absorption or at the gut wall during the process of absorption is still far from decided. Thus Bunyan, Green, Murrell, Diplock & Cawthorne (1968) have suggested that vitamin E may affect the process by which some lipids are absorbed, and Gridgeman (1944) states that even the apparently well-authenticated synergism between dietary vitamins A and E may itself be questionable under certain dietary conditions. These factors are under investigation at present in the hope that they may yield a clearer picture of the vitamin A–vitamin E relationship before and during absorption.

The nature of the interaction between the two vitamins during utilization for growth is similarly still unclear. Green, Muthy *et al.* (1967) gave considerable evidence that vitamin A was not subject to peroxidative destruction in vivo. In Expt 3 we were able methodologically to separate the study of utilization from problems connected with the storage of vitamin A. It was found that the metabolism of a single 50 i.u. dose of radioactive vitamin A, given to the depleted rat and utilized almost completely during the next 6 days, was unaffected by the presence of vitamin E in the tissues. This result seems to exclude any remaining possibility that vitamin E acts in vivo to protect vitamin A from either peroxidation or 'co-oxidation' (Tappel, 1962).

Taken in conjunction with the preceding results, our findings in Expt 4 on liver storage take on some additional significance. Although previously, Green, Muthy *et al.* (1967) had found that vitamin E did not affect the rate of depletion of vitamin A from rat liver, when the total initial reserves were of the order of 3000 i.u., we have now found that vitamin E markedly affects the depletion rate when the initial reserves are of the order of 30000 i.u. in agreement with Moore (1940) and Davies & Moore (1941). It must be noted, once again, that the direction of these results is opposite to that expected if vitamin E were acting as an antioxidant; for, if that were so, the protective effect should be greater at the lower level of vitamin A. This remarkable effect of α-tocopherol was produced by giving only 1 mg per week. The likelihood that it is indeed due to some specific action can be judged in perspective by considering the generally small, often marginal and variable effects of vitamin E on the uptake and storage of vitamin A from normal dietary concentrations or from moderate dosages (Green, Muthy *et al.* 1967; Hickman *et al.* 1944*a, b*; Lemley *et al.* 1947; Harrill, Minarik & Gifford, 1965; Murray & Campbell, 1955; Reber, Morrill, Norton & Rhoades, 1956). The effect of vitamin E in vivo, therefore, would seem to be due to a specific effect in increasing the capacity of the liver to retain vitamin A, especially at high concentrations. This could perhaps be due to an increase in number or size of the Kupffer cells; or it might be due to an effect on membranes in these cells, so limiting their permeability or 'leakage'. It is possible, perhaps, that the effect, observed by Hickman *et al.* (1944*a*) and already referred to, of vitamin E in prolonging the growth response to limited amounts of vitamin A could be mediated through a primary influence on the liberation of vitamin A from the liver; or, in extenso, vitamin E may control, by some similar mechanism, certain tissue concentrations of vitamin A, perhaps by an effect on specific protein synthesis. Deshmukh, Malathi & Ganguly (1964) have suggested that the depletion of vitamin A from the liver might be dependent on the tissue concentrations of special lipoproteins.

It would seem, from the work of Diehl (1964, 1966*a, b*) and of Roels, Guha, Trout, Vakil & Joseph (1964) that the role of vitamin E may be associated with the control of protein synthesis. Dinning and his colleagues have pursued this line of thought for several years and have suggested that vitamin E may have a role in affecting DNA synthesis (Dinning, 1962). Such a role could account for many of the aspects of the vitamin A–vitamin E relationship. It would also correlate many of the other observed effects of vitamin E: as a protein-sparing agent (Hove & Hardin, 1951; Hove, Copeland & Salmon, 1949); as a protective agent against nutritional muscular dystrophy in all species, and especially its specific interaction in sulphur amino acid metabolism in the chick (Scott, 1962); on serum lipoprotein synthesis (Oppenheimer, Shulman, Roberts & Milhorat, 1958; Roels *et al.* 1964); on haemoglobin synthesis in protein-deficient rats (Bencze, Gerlóczy, Ugrai & Kneiszl, 1966); on cell regeneration after partial hepatectomy (Maros, Fodor, Kovacs & Katonai, 1966); on the synthesis of collagen (Brown, Button & Smith, 1967); and on the synthesis of microsomal demethylating enzymes (Carpenter, 1967). It is of interest that the earliest pathological changes in the liver of rats with incipient nutritional hepatic necrosis due to combined selenium and vitamin E deficiency are associated with disturbances in the cell nucleus (Fite,

1954) and that apparently identical nuclear changes are the first detectable signs of silver poisoning in the liver of the vitamin E-deficient rat (P. Grasso, private communication).

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CORRIGENDUM

Milk in schools: an experiment in nutrition education

BY J. C. McKENZIE, JULIET MATTINSON AND JOHN YUDKIN

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Page 813, line 28:

For   about 90 ml   *read* 190 ml

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