ON THE FUNCTION AND METABOLISM OF VITAMIN E
II. THE EFFECTS OF VITAMIN E ON THE LEVEL OF NON-VITAMIN E REDUCING COMPOUNDS PRESENT IN ANIMAL TISSUES*

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Hines and Mattill (1943) using the Emmerie-Engel (1938) assay method for vitamin E in rat tissues showed that the level of vitamin E so determined was related to the level of vitamin E in the diet. This finding has been confirmed and extended for many species. Bolliger and Bolliger-Quaife (1955) reported that chromatography of various tissues gave two Emmerie-Engel reactive compounds of which only one was vitamin E, while more recently chickens raised on vitamin E-free diets containing selenium have been found to contain Emmerie-Engel reactive material which did not chromatograph as vitamin E (Bieri, et al., 1959; Bieri, et al., 1960).

Work on the effect of antioxidants in the replacement of vitamin E has shown that rats can be carried through reproduction or cured following vitamin E deficiency resorption-gestation by certain antioxidants (Markees, 1953; Markees, 1955; Draper, et al., 1956; Draper, et al., 1958; Draper and Johnson, 1958). Over the past three years we have assayed tissues of rats raised on antioxidants and in all cases have found Emmerie-Engel reactive material even in third generation animals on vitamin E-free rations. Chromatographic procedures have proven this not to be vitamin E. In comparing rats fed vitamin E-free synthetic rations containing vitamin E or antioxidants, or fed checkers, it has recently become evident that the amount of this unknown

*Part 1, Alaupovic, P. and Johnson, B. Connor, Arch. Biochem. Biophys., 84, 274 (1959).

reducing compound is related directly to the level of antioxidant (vitamin E or other) in the diet.

Animals used in these experiments were male rats maintained on checkers and female rats (Sprague-Dawley strain) maintained on a vitamin E free diet (Draper, et al., 1958) treated with α-tocopherol at various levels or with N,N'-diphenyl-p-phenylenediamine (DPPD), depletion of vitamin E in the female rats having been assured by carrying them through a resorption-gestation. All animals in groups 1 to 5 (Table I) were fed the vitamin E-free diet for six months following resorption-gestation. The α-tocopherol treated animals were then given the doses indicated in Table I. The animals were killed 5 days after treatment on the basis of the short-time required for cure in the rat-curative assay. The DPPD animals were given the DPPD containing diet for two weeks prior to assay. The checker fed male rats were approximately eight months old.

For assay, homogenized livers were extracted with foaming solvent (Moore, et al., 1941), neutral fats were precipated at low temperature and vitamin A and carotenoids were removed on Florex XXS columns. Total vitamin E plus other reducing substances were determined on the eluates by the Emmerie-Engel reaction and the mean data are given in Table I. Non-vitamin E reducing compounds in these eluates were separated from vitamin E by the paper chromatographic procedure of Eggitt and Ward (1953), the chromatograms being developed with 75% ethanol. Using this chromatographic procedure the Florex column eluates gave Emmerie-Engel positive spots for vitamin E (R_f 0.25) when present, for DPPD (R_f 0.83) when present, and for another compound (R_f 0.00) which was always present. When this origin spot was eluted and rechromatographed on Eggitt and Ward paper, but developed with 80% n-propanol, two Emmerie-Engel reactive compounds were obtained (R_f's 0.40, and 0.53 for the main component). The spot at R_f 0.40 was found to be acid reduced coenzyme Q_{10} (chromane form) which was not found when the extracts were not passed through the Florex column and in separate experiments was found to be formed from coenzyme Q_{10} by the Florex column. The non-vitamin E Emmerie-Engel reactive

material previously reported (Bolliger, et al., 1955) appears to have been due to the presence of reduced coenzyme Q_{10} or Q_9 formed by the Florex columns used, while the non-vitamin E compound reported here was probably included in the vitamin E spot obtained by Bolliger et al. (1955), since in the system they used (Green, et al., 1955) the R_f's are quite similar for vitamin E and the unknown (R_f 0.75 for α-tocopherol and 0.78 for the unknown).

For quantitative assay of the individual reducing compounds in the livers of the animals the α-tocopherol spot and similarly the DPPD spot from the 75% ethanol chromatograms were eluted and assayed with Emmerie-Engel reagent and the new compound was assayed following elution of the 0.53 spot from the 80% n-propanol chromatograms. The data are given in Table I.

Table I

Diet	Treatment	No. of Animals	Emmerie-Engel Reactive Material mg/kg Liver Total	Vit. E	Non Vit. E	DPPD
1. E-free	none	4	9.0	n.d.	9.0	-
2. E-free	α-tocopherol[1] 500 mg	5	105.0	75.0	25.0	-
3. E-free	α-tocopherol[2] 500 mg	5	223.0	141.0	54.0	-
4. E-free	DPPD 0.005% of diet	5	63.0*	n.d.	-	-
5. E-free	DPPD 0.005% of diet	2	-	n.d.	54.0	90.0
6. Checkers	none	6	22.0	n.d.	22.0	-

n.d. - not detected

[1]100 mg/rat/day for 5 days

[2]500 mg/rat, 5 days prior to assay

*These extracts are all put through Florex columns to remove vitamin A. This absorbent has been found to also remove 80% of the DPPD. Therefore this figure represents all the unknown plus 20% of the DPPD in the liver.

In addition to the R_f's of 0.00 and 0.53 found for this unknown compound in the systems discussed above an R_f of 0.78 was obtained in the system of Lester and Ramasarma (1959) when developed with 80% n-propanol. Small amounts of this compound have been prepared from such chromatograms, the purest preparations showing absorption peaks at 274 and 330 mμ. It was found that this Emmerie-Engel reactive compound was not identical with other tocopherols,

coenzyme Q_{10} or Coenzyme Q_9 hydroquinones (Lester, et al., 1959), ubichromenol or so called SC compound (Cunningham, et al., 1959), solanochromene (Rowland, 1958) or a new antioxidant isolated from yeast (Forbes, et al., 1958). Cunningham et al. (1959) have reported the presence of substance SB in rat and pig livers with absorption peaks at 274 and 330 mμ which may be similar to or identical with this unknown compound isolated from rat liver.

The data in Table I indicate that much of the reducing material previously determined as vitamin E is, in fact, another compound. This compound was found to be present in all animals, even in those receiving no vitamin E or antioxidant or selenium, however the amount present in the liver appears to be directly related to the presence of antioxidant or vitamin E in the diet and in the animal.

It seems possible that the main role of α-tocopherol and other biologically active antioxidants is in the preservation of this compound formed by the animal body. Work is in progress on the identification and biological importance of this compound.

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