THE EFFECT OF VITAMIN E, VITAMIN A, AND SOME OTHER DIETARY FACTORS ON UBIQUINONES AND UBICHROMENOLS IN THE RAT

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We have investigated the effect of vitamin E and certain other substances on ubiquinone and ubichromenol levels in the rat and wish to record some preliminary findings. Male and female rats, in groups of 12, were studied separately at ages between 2 and 15 months. Twenty different diets were used to study deficiencies of and supplementation with vitamin E, vitamin A, pantothenic acid, riboflavin, thiamin, and selenium, and, in addition, the effect of double deficiencies of selenium and vitamin E and of vitamins A and E. In further experiments, other groups of rats on the various deficient diets were administered with single doses of the missing dietary factors and killed 48 hours later; to minimise variation, grouped litter-mate controls were used in these dosage experiments. The animals remained on test for different lengths of time, varying from 4 weeks for the thiamine groups to 6-15 months for the vitamin E groups. After each experiment, the rats were killed, and similar organs within each group were pooled. In most groups concerned with vitamin E, 14 tissues were examined:- heart, liver, kidney, skeletal muscle, spleen, sciatic nerve, brain, adrenal, lung, fat, uterus, testis, red blood cells and serum. In other groups, a selection of these tissues was made. The tissues were analysed for tocopherols, vitamin A, ubiquinone and ubichromenol by the methods described for some small quantities of these substances by Diplock, Green, Edwin and Bunyan (1960 a). The following results have emerged and will be reported in detail elsewhere.

1. Neither deficiency of nor supplementation with thiamin or riboflavin

affected ubiquinone/ubichromenol levels in the rat (4 organs studied: liver, heart, kidney and spleen). In pantothenic acid deficiency, however, contrary to the findings of Aiyer et al. (1959), liver ubiquinone was increased after 6 weeks to 146 µg./g., compared to 83 µg./g. for supplemented controls. Administration of calcium pantothenate (6 mg.) to deficient animals 48 hours before death reduced the level to 108 µg./g. Similar but only marginally significant effects were found in heart (157 µg./g. increased to 163 µg./g., reduced again to 155 µg./g. after supplementation). Increases in the ubichromenol contents of these organs were also observed in pantothenic acid deficiency and were reversed by supplementation with the vitamin.

2. Vitamin A deficiency had no effect on either ubiquinone or ubichromenol in any tissue studied except liver, which, with regard to vitamin A, is subject to special consideration, and where the results could, in any case, be interpreted on the basis of vitamin E changes. Thus vitamin A deficient livers contained 159 µg./g. of ubiquinone, compared with 117 µg./g. for controls (ubichromenol contents were 71 and 51 µg./g., respectively); since the mean liver weight only decreased by 12% during the 8 weeks test period, this represented an overall increase of ubiquinone in the organ (compare Lowe, Morton and Harrison, 1953, Moore and Sharman, 1959, and Gloor and Wiss, 1959). The vitamin E content of the liver increased from 28 to 44 µg./g. during the deficiency period, whilst in other tissues no change occurred. Furthermore, dosage with 1,600 i.u. of vitamin A 48 hours before death did not affect ubiquinone, ubichromenol or vitamin E levels in the livers of deficient animals, although the vitamin A level increased from 24 to 240 µg./g.

3. Vitamin E deficiency markedly depressed ubiquinone levels in 13 tissues, compared with those of stock animals and tocopherol-supplemented controls (serum did not contain ubiquinone). Ubichromenol levels were also depressed in most tissues. Administration of 2-10 mg. of α-tocopherol acetate to deficient animals 48 hours before death resulted in an increase of ubiquinone in all 13 tissues, raising the levels to or higher than those found in stock animals. Typical increases in the 6 month-old female rat were: uterus, 17 to 67 µg./g.;

sciatic nerve, 10 to 61 μg./g.; heart, 87 to 159 μg./g.; adrenal, 81 to 130 μg./g.; liver 90 to 114 μg./g. A highly significant feature of the vitamin E dosage experiment was that, in every tissue, increase of ubiquinone was accompanied by a marked reduction in ubichromenol. For example, liver decreased from 53 to 23 μg./g., adrenal from 72 to 44 μg./g., and heart from 20 to 9 μg./g. The action of tocopherol is not readily attributable to a "protective" or "anti-oxidant" effect, for it would be difficult to account for the significant ubiquinone-ubichromenol inverse relationship on such a basis. Furthermore, vitamin A levels in the 13 tissues only slightly increased by tocopherol administration, reinforcing the concept that vitamin A has little influence on tissue ubiquinone if vitamin E is present. These findings on the action of vitamin E conflict with those of Moore (1959) and Morton and Phillips (1959).

4. Compound vitamin A/E deficiency depressed ubiquinone in liver (and sometimes heart) to exceptionally low levels, other organs being little affected. Liver (male, 4 months) contained 44 μg./g.; compared with over 100 μg./g. for normal tissue. Administration of vitamin A 48 hours before death decreased ubiquinone to 15 μg./g., while vitamin E (10 mg.) increased it to 71 μg./g.

5. Sodium selenite (0.05 to 0.01 μg./g.), added to necrogenic diets (deficient in Factor 3) and to ordinary E-deficient diets, stimulated ubiquinone synthesis in the liver (increase from 101 μg./g. for E-deficient liver at 3 months to 233 μg./g. after selenium supplementation for 2 weeks).

6. All the organs of the female rat contained significantly higher amounts of ubiquinones, ubichromenols, tocopherols and vitamin A than corresponding organs in the male.

7. In preliminary experiments, the addition of DPPD (0.05%)or 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (Santoquin: 0.015%) to vitamin E-deficient diets was found not to affect ubiquinone/ubichromenol levels in heart or liver, although such amounts have been shown by other workers to ameliorate or prevent certain symptoms of the deficiency state.

Massive doses 48 hours before death had no effect in heart, although there was a significant increase in liver when 40 mg. of Santoquin were administered. These experiments appear to suggest a possible difference between the action of vitamin E and the non-physiological antioxidants, but further work, now in progress, will determine whether the effect of the latter substances is influenced by levels of vitamin A in the diet. It is already clear that caution is necessary in interpretation unless the interlocking vitamin E/A relationships in the tissues are worked out. In this respect, liver is not perhaps the most suitable organ for study. It is possible that the difference between our findings and those of Morton and Phillips may be based on the fact that our vitamin E-deficient diet contained far more vitamin A than theirs did.

8. In several of the above investigations we have obtained evidence in support of the natural occurrence of ubichromenol by the qualitative picture of ubiquinone-ubichromenol relationships under varying conditions and by recovery experiments with both ubiquinone and ubichromenol. Draper and Csallany (1960) have recently suggested that ubichromenol is an artefact produced by heating ubiquinone with ethanolic alkali. We have found that, in the absence of pyrogallol, and under the conditions described by these authors, a reducing substance with spectral characteristics somewhat similar to those of ubichromenol is indeed formed; but that the substance can be easily distinguished by paper chromatography from ubichromenol, which, as we have previously suggested (Green et al. 1960), appears to require an acid-catalysed reaction to produce it. The recent findings of Crider et al. (1960) are also relevant. The substances (Rf 0.40 and 0.53) described by these authors may well be ubichromenols 45 and 50, respectively (Diplock et al. 1960 b), but adequate purification is necessary to reveal the existence of the characteristic inflexion at 282 mμ in their spectra. We have found that columns of the activated Florex and Floridin types are too destructive to be relied on for the analysis of ubiquinones and ubichromenols.

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