# Simultaneous determination of tissue tocopherols, tocotrienols, ubiquinols, and ubiquinones

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**Abstract** A tissue-specific distribution of the various vitamin E forms, tocotrienols and tocopherols, has been found, suggesting that these forms have unique roles in cellular functions. A sensitive procedure is described for the simultaneous determination of individual tocopherols, tocotrienols, ubiquinols, and ubiquinones using gradient high pressure liquid chromatography (HPLC) and electrochemical detection for vitamin E homologues and ubiquinols, and in-line UV detection for ubiquinones. Using this method, the lipophilic antioxidant complement of a variety of hairless mouse tissues was analyzed. Of the vitamin E forms, brain contained virtually only α-tocopherol (5.4 ± 0.1 nmol/g; 99.8%) and no detectable tocotrienols were found. By contrast, skin contained nearly 15% tocotrienols and 1% γ-tocopherol. In other tissues, the α-tocopherol content was higher (20 nmol/g), while each of the other forms represented about 1% of the total (γ-tocopherol 0.2 to 0.4 nmol/g, α-tocotrienol 0.1, γ-tocotrienol 0.2). Ubiquinol-9 concentrations were highest in kidney (81 nmol/g) and in liver (42 nmol/g), while the highest ubiquinone-9 concentrations were found in kidney (301 ± 123 nmol/g) and heart (244 ± 22 nmol/g). Liver contained nearly identical concentrations of each of the redox couple (ubiquinol-9 (41 ± 16 nmol/g) and ubiquinone-9 (46 ± 18 nmol/g). The unique distribution of these various antioxidants in the tissues measured suggests their distribution may be dependent upon selective mechanisms for maintaining antioxidant defenses in each tissue.—**Podda, M., C. Weber, M. G. Traber, and L. Packer.** Simultaneous determination of tissue tocopherol, tocotrienols, ubiquinols, and ubiquinones. *J. Lipid Res.* 1996. **37:** 893–901.

**Supplementary key words** vitamin E • coenzyme Q • HPLC • electrochemical detection • tissue • method

The unique distribution of various antioxidants in tissues suggests their distribution may be dependent upon selective mechanisms for maintaining antioxidant defenses. Plasma α-tocopherol concentrations, for example, are maintained through the action of the hepatic α-tocopherol transfer protein (1) and genetic defects in this protein result in severe vitamin E deficiency (2–4). However, little is known about cellular mechanisms that regulate tissue lipophilic antioxidant concentrations. This information is especially important because hydrophilic antioxidants have roles in cellular functions in addition to their antioxidant functions (5).

Coenzyme Q (Q; reduced form: ubiquinol, oxidized form: ubiquinone) functions as electron carrier in mitochondria (6). However, it is also present in considerable amounts in endoplasmic reticulum, Golgi apparatus, lysosomes, peroxisomes, and plasma membranes (7). This broad distribution in intracellular organelles emphasizes that Q may play an antioxidant role in membranes (8, 9). Indeed, ubiquinol protects against lipid peroxidation more efficiently than α-tocopherol in low density lipoproteins (10–12).

The major lipophilic antioxidant in plasma, membranes, and tissues is vitamin E (1). Vitamin E is the collective name for the eight naturally occurring molecules (four tocopherols and four tocotrienols), that exhibit vitamin E activity (**Fig. 1**). Tocotrienols differ from tocopherols in that they have an isoprenoid instead of a phytyl side chain; the four forms of tocopherols and tocotrienols differ in the number of methyl groups on the chromanol nucleus (α- has 3, β- and γ- have 2, while δ- has 1).

A very sensitive method of detection is required for the quantitation of each of these lipophilic antioxidants to determine their roles in protecting tissues against oxidative damage. This is especially important for tocotrienols because they are present at low concentrations, but may exert substantial antioxidant effects (13–15). HPLC separation is required for analysis of vitamin E, but ultraviolet (UV) detection does not pro-

Abbreviations: BHT, butylated hydroxytoluene; EC, electrochemical; EDTA, ethylenediaminetetraacetic acid; HPLC, high pressure chromatography; Q, enzyme Q (reduced form: ubiquinol; oxidized form: ubiquinone); UV, ultraviolet; SDS, sodium dodecyl sulfate.

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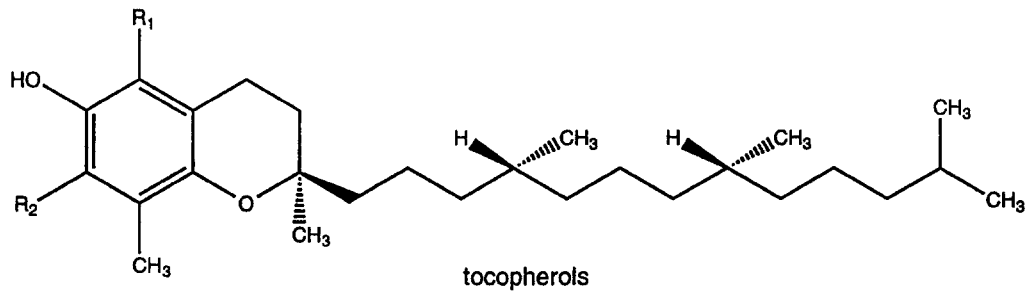
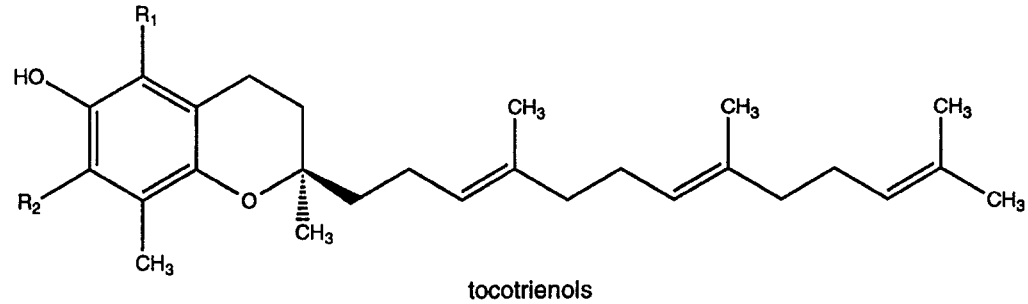
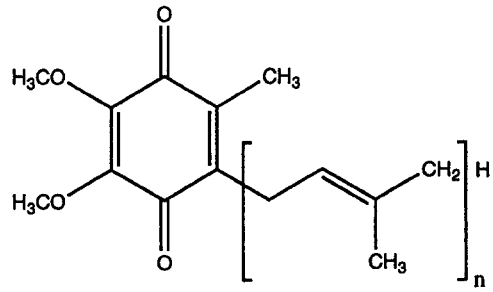


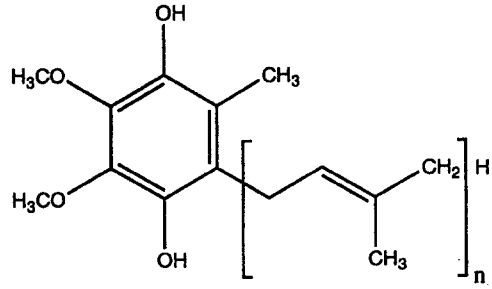

tocopherols




tocotrienols




ubiquinone




ubiquinol

**Fig. 1.** Structures of tocopherols, tocotrienols, ubiquinol, and ubiquinone. For tocopherols and tocotrienols: α, $R_1$ and $R_2$ = $CH_3$; β, $R_1$ = $CH_3$, $R_2$ = H; γ, $R_1$ = H, $R_2$ = $CH_3$; δ $R_1$ = H, $R_2$ = H. For ubiquinone and ubiquinol: n can equal 9 or 10. Ubiquinone/ubiquinol-9 is the predominant form in mice and rats, while ubiquinone/ubiquinol-10 is the predominant form in guinea pigs and humans.

vide sufficient sensitivity. The simultaneous determination of tocopherols and ubiquinol in biological tissue using electrochemical detection (EC) with in-line UV detection for ubiquinone determination has been described previously by our laboratory (16). However, separation of the different tocopherols and tocotrienols

was not accomplished. By contrast, an HPLC/electrochemical (EC) method that efficiently separates and detects various vitamin E homologues (17) is not suitable for ubiquinol/ubiquinone separation due to the extremely prolonged retention times of these highly lipophilic compounds.

We describe herein a very sensitive procedure for the simultaneous determination of individual tocopherols, tocotrienols, ubiquinols, and ubiquinones using gradient HPLC and electrochemical detection for vitamin E homologues and ubiquinols with in-line UV detection for ubiquinones. The method is demonstrated to be effective for the measurement of these lipophilic antioxidants in several mouse tissues, including liver, kidney, heart, brain, and skin.

## METHODS AND MATERIALS

### Materials

Highest purity solvents and reagents were used. The reagent alcohol consisted of 2-propanol–ethanol 5:95 (v/v) (Fisher Scientific, Pittsburgh, PA). Lithium perchlorate was obtained from Johnson Matthey, Ward Hill, MA). Sodium dodecyl sulfate (SDS) and sodium dithionite were obtained from Fisher, butylated hydroxy toluene (BHT) from Sigma (St. Louis, MO). Ubiquinone-9 and 10 standards were a gift from Nisshin Flour Milling Co., Ltd. (Tokyo, Japan). Tocopherol standards were kindly provided from Henkel (La Grange, IL). Tocotrienols were purified from palm oil tocotrienol-rich fraction (TRF) by Dr. Asaf A. Qureshi.

### Preparation of standards

Individual standards of α- and γ-tocopherols, α- and γ-tocotrienols, ubiquinol 9 and 10, and ubiquinone 9 and 10 were diluted in ethanol to obtain stock solutions between 50 and 200 μM; the exact concentration was determined spectrophotometrically (**Table 1**). The ubiquinol standards were prepared as described (16). Briefly, a stock solution of ubiquinone (50–150 μM) in ethanol was reduced with 100 mg of sodium dithionite in 3 ml water at room temperature in the dark for 30 min. The ubiquinols were extracted with hexane, dried under nitrogen, and resuspended in ethanol. The concentration of the resulting stock solution was determined spectrophotometrically using the molar extinction coefficient for ubiquinol-9 or ubiquinol-10 (Table 1). The stock solution kept at -200°C (stable for months).

The working standards were obtained by mixing appropriate amounts of the stock solutions. The working standards used for repeated determinations were stored in a cooled autoinjector (4°C) for a maximum of 4 h. The gradual oxidation of solutions of ubiquinols 9 and 10 was found to be less than 6% after 4 h under these conditions.

### Animals

All experiments and procedures for animal handling were approved by the Animal Care and Use Committee of the University of California, Berkeley. Female hairless mice (SKH1, 8–12 weeks old) were purchased from Charles River Laboratories (Wilmington, MA) and were kept under standard light and temperature conditions. Food (Harlan Teklad Rodent Diet 8656, Madison, WI) and water were provided ad libitum. Three mice were anesthetized, killed by cervical dislocation, and the tissues were removed, rinsed with phosphate-buffered saline, blotted dry, and an aliquot was frozen in liquid nitrogen.

### Processing of samples

All tissues were extracted as described by Burton, Webb, and Ingold (18), with the exception of skin samples, which were handled as follows. Approximately 50 mg frozen skin was weighed, ground under liquid nitrogen, homogenized with a Teflon pestle for 1 min on ice in a 10-ml Potter-Elvehjem tube containing 50 μl BHT (10 mg/ml) and 2 ml of skin buffer (130 mM NaCl, 1 mM disodium EDTA, 10 mM sodium phosphate, pH

TABLE 1. Physicochemical data of tocopherols, tocotrienols, ubiquinols, and ubiquinones

| Substance | MW | λmax [nm] | E1%, 1 cm[a] | ε[a] | Reference |
|---|---|---|---|---|---|
| α-Tocopherol | 430.7 | 292 | 75.8 | 3270 | (36) |
| β-Tocopherol | 416.7 | 296 | 89.4 | 3730 | (36) |
| γ-Tocopherol | 416.7 | 298 | 91.4 | 3810 | (36) |
| δ-Tocopherol | 402.7 | 298 | 87.3 | 3520 | (36) |
| α-Tocotrienol | 424.7 | 292 | 91.0 | 3870 | (36) |
| β-Tocotrienol | 410.7 | 295 | 87.5 | 3600 | (36) |
| γ-Tocotrienol | 410.7 | 298 | 103.0 | 4230 | (36) |
| δ-Tocotrienol | 396.7 | 292 | 83.0 | 3300 | (36) |
| Ubiquinol 9 | 796.6 | 290 | 51.7 | 4120 | (37) |
| Ubiquinol 10 | 864.7 | 290 | 46.4 | 4010 | (37) |
| Ubiquinone 9 | 794.6 | 275 | 185 | 14700 | (37) |
| Ubiquinone 10 | 862.7 | 275 | 165 | 14240 | (37) |

[a]Extinction coefficients are given for ethanol solutions.

7.0). The homogenate was transferred to a screwcap tube and 1 ml 0.1 M SDS was added and mixed vigorously for 30 sec. After addition of 2 ml ethanol, brief mixing and sonication, the homogenates were extracted with 2 ml hexane and an appropriate aliquot was dried down under nitrogen and resuspended in ethanol–methanol 80:20 and injected onto the HPLC column.

The vitamin E content of mouse chow was determined after saponification because the diet contains added tocopheryl acetate. Briefly, 1 g chow was mixed vigorously with 5 ml 1% w/v aqueous ascorbic acid. After addition of 10 ml ethanol and 1.5 ml saturated KOH, the samples were saponified at 70°C for 30 min, protected from light. After cooling on ice and addition of 5 ml water, the samples were extracted with 10 ml hexane. Five ml of the upper hexane layer was collected and taken to dryness under nitrogen. The residue was diluted 25-fold and injected onto the HPLC.

### HPLC analysis

The HPLC system consisted of a Hewlett-Packard 1050 series gradient pump, an SCL-10A Shimadzu system controller with an SIL-10A autoinjector with sample cooler, a Beckman Ultrasphere ODS C-18 column, 4.6 mm i.d., 25 cm, 5 µm particle size with a Brownlee Spheri-5 RP-18 precolumn 5 micron 30 × 4.6 mm, a Hewlett-Packard 1050 diode array detector, and a Bioanalytical Systems LC-4B amperometric electrochemical detector with a glassy carbon electrode. The detectors were setup in line, the eluent passing first through the diode array detector. The delay between UV and EC detectors was 2 sec.

A gradient was used consisting of a mixture of A (methanol–water (80:20 (v/v) and 0.2% (w/v) lithium perchlorate) and B (ethanol with 0.2% (w/v) lithium perchlorate) at a flow rate of 1 ml/min. The initial conditions were 39% A and 61% B. After 16 min the mobile phase was changed linearly over 2 min to 100% B; then 100% B was continued for 10 min, after which the system reverted linearly over 2 min to the initial conditions (39% A and 61% B). Each run lasted 40 min. The diode array detector was set to 275 nm for detection of ubiquinones; the electrochemical detector was in the oxidizing mode, potential 500 mV, full recorder scale at 50 nA. Data were collected with a Perkin Elmer Interface and Turbochrom Software (P. E. Nelson, Cupertino, CA).

### Recovery and reproducibility

Liver (100 mg) was homogenized as described above. The homogenate was divided into two equal aliquots, 850 pmol of each α- and γ-tocotrienols were added to one aliquot. Both samples were then extracted as described above. The percent recovery was calculated by subtracting the amount of tocotrienols measured in liver from the amount found in liver with added internal standards and multiplied by 100.

### Statistical analyses

The differences in vitamin E and ubiquinone/ubiquinol contents in the mice were analyzed using one-way ANOVA. All statistical analysis was carried out using SuperAnova for the Macintosh (Berkeley, CA). A *P*-value < 0.05 was considered statistically significant.

## RESULTS

### HPLC separation

Individual antioxidants were well separated from a mixture containing α-tocopherol, γ-tocopherol, α-tocotrienol, γ-tocotrienol, ubiquinol-9, ubiquinol-10, ubiquinone-9, and ubiquinone-10 (**Fig. 2**). The HPLC separation requires changing the composition of mobile phase mid-run; the linear gradient is initiated at 16 min

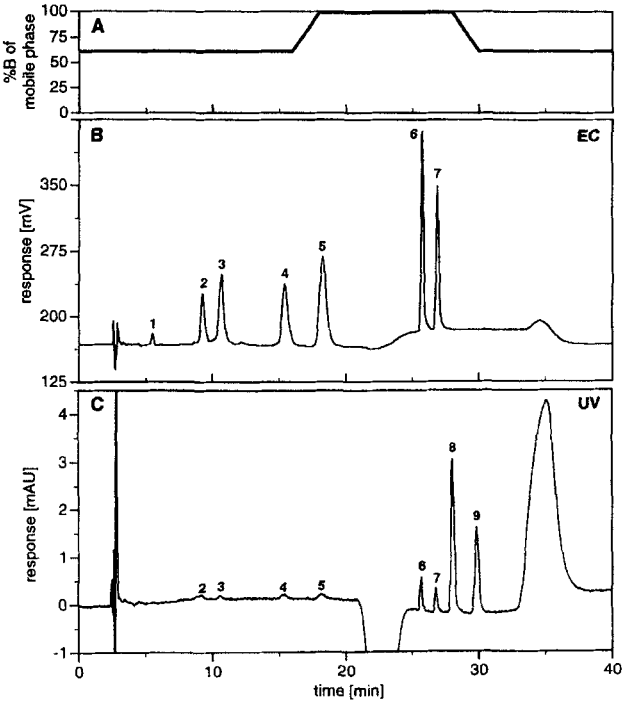



**Fig. 2.** Chromatogram of standard compounds. Shown are: A, gradient scheme; B, detection by EC of known compounds: BHT [1], γ-tocotrienol [2], α-tocotrienol [3], γ-tocopherol [4], α-tocopherol [5], ubiquinol-9 [6], and ubiquinol-10 [7]; C, UV detection at 275 nm of ubiquinone-9 [8] and ubiquinone-10 [9]. The standards of γ-tocotrienol [2], α-tocotrienol [3], γ-tocopherol [4], α-tocopherol [5], ubiquinol-9 [6], and ubiquinol-10 [7] are also visible at this wavelength. Note peaks of compounds detected by both UV and EC are present in the UV tracing 2 sec prior to detection by EC.

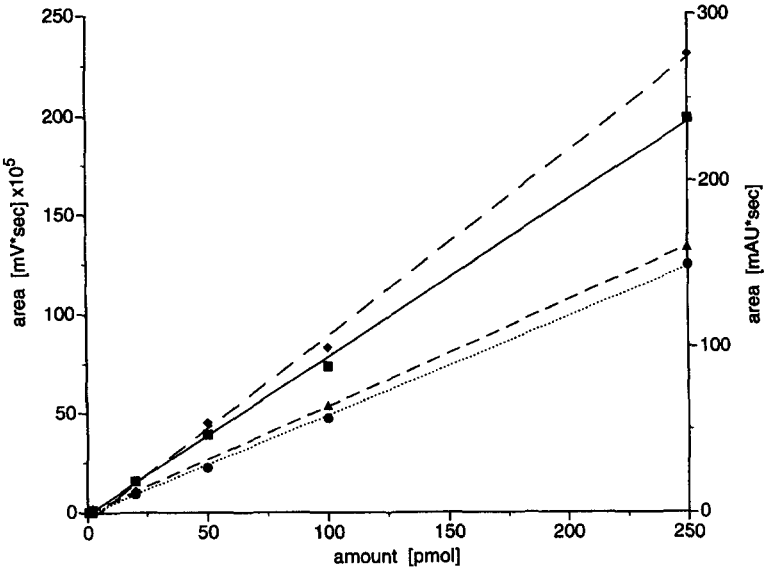



**Fig. 3.** Linear responses to known quantities of a mixture of lipophilic antioxidants. A typical standard curve generated from the EC detection of pmol amounts ubiquinol-9 (▲), α-tocopherol (■), and γ-tocopherol (●) (left axis) and from the UV-detection of ubiquinone 9 (◆, right axis).

and is shown as %B mobile phase in Fig. 2A. This change in mobile phase perturbs the baseline both during EC and UV detection at 21 to 24 min and at 33 to 37 min. The output from the EC detector (Fig. 2B) demonstrates the separation of tocopherols, tocotrienols, and ubiquinols, which elute in the opposite order of their lipophilicity. The vitamin E forms elute as follows: γ-tocotrienol, α-tocotrienol, γ-tocopherol, then α-tocopherol. Subsequently, ubiquinol-9 and ubiquinol-10 are eluted as sharp peaks as ethanol forms an increasing proportion of the mobile phase. Ubiquinone-9 and ubiquinone-10 are eluted last and are detectable only by the in-line UV-diode array detector (Fig. 2C). Note that the UV-detector physically precedes the EC detector, so that peaks of compounds that are detected by both are detected 2 sec earlier by the UV-detector.

### Standard curves

Standard curves for ubiquinol-9, α- and γ-tocopherols quantitated from the output of EC-detector and for ubiquinone-9 from the UV-detector are shown in **Fig. 3.**

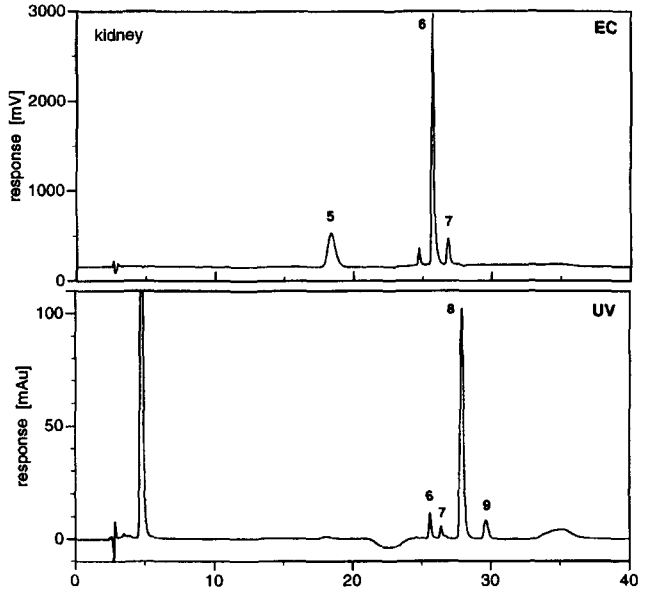



**Fig. 4.** Chromatogram of kidney lipophilic antioxidants. Shown is a typical chromatogram of the antioxidants detected by EC (upper panel) and by UV (275 nm; lower panel).

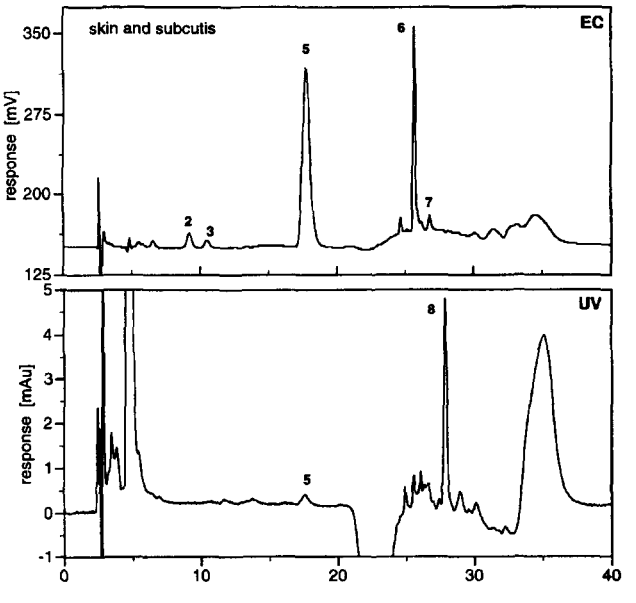



**Fig. 5.** Chromatogram of skin with subcutis. Shown is a typical chromatogram of the antioxidants detected by EC (upper panel) and by UV (275 nm; lower panel).

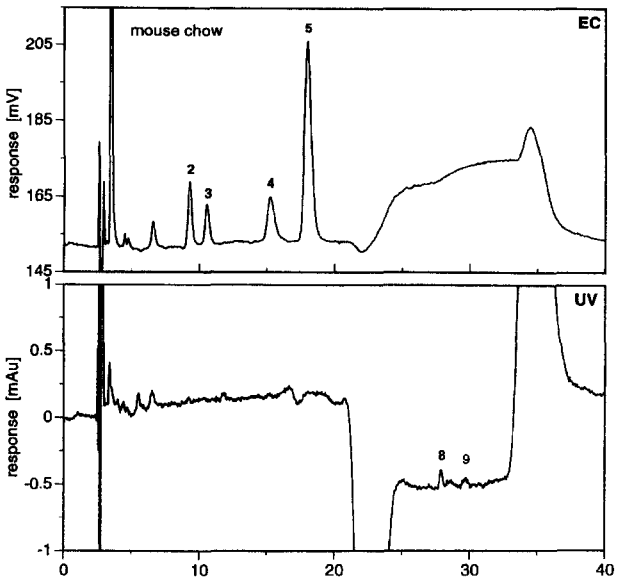



**Fig. 6.** Chromatogram of mouse chow. Shown is a typical chromatogram of the antioxidants detected by EC (upper panel) and by UV (275 nm; lower panel).

Responses were linear from 0.5 to 250 pmol for each of these compounds.

### Recovery and detection limits

The recoveries of α-tocopherol, ubiquinols 9 and 10, and ubiquinones 9 and 10 from rat liver using the described extraction procedure has been previously reported by our laboratory to be > 90% (16). Using the present method we found that the recovery of added γ-tocopherol was 93%, α-tocotrienol 85%, and γ-tocotrienol 94%.

The limit of electrochemical detection was 0.1 pmol for tocopherols and 0.3 pmol for ubiquinols. The limit of UV-detection was 0.2 pmol for ubiquinone. The high extinction coefficient allows for detection of small amounts of ubiquinone.

### Tissue antioxidant concentrations

Representative chromatograms from kidney (**Fig. 4**), skin (**Fig. 5**) and mouse chow (**Fig. 6**) demonstrate the separation of these lipophilic antioxidants by this HPLC method. After 40 min, no peaks were detectable at 206 nm; thus, the lipids eluted completely from the column and no post-run column wash-out was required before the next injection. In fact, a large series of samples (> 100) were run without post-run time or column cleaning.

The concentrations of the various vitamin E homologues in tissues and in diet are shown in **Table 2.** In all tissues, α-tocopherol represents the major form of vitamin E. It is striking that the brain contains virtually only α-tocopherol (99.8%, **Table 3**). Although both the brain and skin contain similar α-tocopherol concentrations, the skin contains nearly 15% tocotrienols, but only 1% γ-tocopherol. The associated subdermal fat was not the source of these vitamin E forms because in skin samples which had the fat removed, the tocotrienol concentrations were actually higher (Table 2, compare skin with skin and subcutis). In other tissues (heart, kidney, liver), each of these forms represents about 1% of the total.

A wide range of coenzyme Q concentrations in the various tissues is also shown in Table 2. Ubiquinol concentrations were highest in kidney (81 nmol/g) and in liver (42 nmol/g). The liver was unique in that ubiquinol-9 and ubiquinone-9 concentrations were nearly identical. Skin and brain had similar concentrations of both ubiquinols and ubiquinone 9; with ubiquinols representing around 20% of the total coenzyme Q. The highest ubiquinone-9 concentrations were found in heart and kidney, which contain 5- to 10-fold higher amounts than did the other tissues. These ubiquinone concentrations were far greater than their respective ubiquinol concentrations.

## DISCUSSION

Using a simple reliable method for the separation and quantitation of the major lipophilic antioxidants, this investigation demonstrates a unique distribution of tocopherols and tocotrienols and of coenzyme Q in tissues. The simultaneous measurement of these compounds allows complete assessment of the lipophilic

TABLE 2. Lipophilic antioxidants in hairless mouse tissue (nmol/g tissue) and mouse chow (mg/kg diet)

| Tissue | α-Tocopherol | γ-Tocopherol | α-Tocotrienol | γ-Tocotrienol | Ubiquinol-9 | Ubiquinol-10 | Ubiquinone-9 | Ubiquinone-10 |
|---|---|---|---|---|---|---|---|---|
| Brain | 5.4 ± 0.1 | 0.01 ± 0.02 | n.d. | n.d. | 1.6 ± 0.1 | 0.6 ± 0.1 | 10.2 ± 0.5 | 3.4 ± 0.5 |
| Heart | 24.2 ± 1.1 | 0.19 ± 0.05 | 0.08 ± 0.01 | 0.19 ± 0.05 | 19 ± 4 | 2.8 ± 0.7 | 245 ± 22 | 21 ± 8 |
| Kidney | 21.9 ± 0.6 | 0.35 ± 0.06 | 0.06 ± 0.04 | 0.15 ± 0.07 | 81 ± 29 | 11 ± 6 | 302 ± 124 | 31 ± 14 |
| Liver | 21.2 ± 2.9 | 0.29 ± 0.03 | 0.10 ± 0.04 | 0.19 ± 0.16 | 42 ± 16 | 1.7 ± 0.3 | 46 ± 18 | n.d. |
| Skin | 5.4 ± 1.6 | 0.04 ± 0.00 | 0.24 ± 0.20 | 0.76 ± 0.71 | 2.2 ± 0.3 | 0.4 ± 0.0 | 7.6 ± 1.9 | n.d. |
| Skin and subcutis | 5.7 ± 0.2 | 0.03 ± 0.01 | 0.14 ± 0.02 | 0.41 ± 0.10 | 2.6 ± 0.5 | 0.5 ± 0.1 | 8.1 ± 2.5 | n.d. |
| Chow | 30 ± 6 | 10 ± 1 | 3.1 ± 0.7 | 7.4 ± 1.7 | | | | |

n.d., not detectable.

TABLE 3. Percent distribution of vitamin E homologues in hairless mouse tissues

| Tissue | α-Tocopherol | γ-Tocopherol | α-Tocotrienol | γ-Tocotrienol |
|---|---|---|---|---|
| Brain | 99.8 ± 0.4 | 0.2 ± 0.4 | – | – |
| Heart | 98.1 ± 0.2 | 0.8 ± 0.2 | 0.3 ± 0.0 | 0.8 ± 0.2 |
| Kidney | 97.5 ± 0.6 | 1.6 ± 0.3 | 0.3 ± 0.2 | 0.6 ± 0.3 |
| Liver | 97.2 ± 1.5 | 1.4 ± 0.3 | 0.5 ± 0.3 | 0.9 ± 0.9 |
| Skin | 85.6 ± 7.2 | 0.6 ± 0.3 | 3.4 ± 1.5 | 10.4 ± 6.0 |
| Skin + subcutis | 90.8 ± 1.4 | 0.5 ± 0.2 | 2.2 ± 0.3 | 6.5 ± 1.3 |

antioxidant defense of a tissue, which has not been previously measured in a single aliquot of tissue. These lipophilic antioxidants readily scavenge peroxyl radicals and thus prevent lipid, protein, and DNA oxidation (1, 8). The data presented here further suggest that tissues have specific mechanisms for accumulating different amounts of these antioxidants. This may reflect the capability of various tissues to select the most effective antioxidants or that these lipophilic compounds have unique functions in the various tissues.

The very different distribution of the various vitamin E forms in different organs (Table 2) suggests that these may be independently regulated in each tissue. Brain vitamin E content is especially noteworthy because brain was found to contain only α-tocopherol. The vitamin E content of brain is spared in response to a vitamin E-deficient diet (19–24) and does not markedly increase in response to a vitamin E-supplemented diet (25). Furthermore, feeding a diet deficient in α-tocopherol, but supplemented with γ-tocopherol, did not markedly enrich the brain with γ-tocopherol (26). Taken together these data suggest that there may be a specific transport mechanism for α-tocopherol through the blood/brain barrier.

Not only do the various vitamin E homologues have different antioxidant capabilities, they may have important physiological roles beyond their antioxidant activities (5). For example, skin has been suggested to be an important storage site for vitamin E and a major excretory organ for this vitamin (27). However, skin vitamin E could also have a regulatory role in maintaining barrier function. Skin contains a high proportion of tocotrienols, which could inhibit cholesterol synthesis (14). This is important because cholesterol is a key component of the lipid barrier of the stratum corneum (28). In addition, vitamin E may enhance penetration and resorption of skin lipids (29). Taken together these data suggest complex regulatory mechanisms for maintenance of skin vitamin E content and composition.

It should be noted that the tissues analyzed were obtained from mice that had been fed standard mouse chow, and were not fed diets specifically enriched in unique vitamin E forms. The presence of both α- and γ-tocotrienols suggests that the diet contains palm oil, a major source of these vitamin E forms (30, 31). Although the vitamin E content of the diet from only one manufacturer was measured, it is possible that other diets based on natural components also have mixtures of vitamin E forms. Therefore, various forms of vitamin E might be expected in mice consuming chow diets.

Measurement of the ubiquinol/ubiquinone contents of the tissues also provides important information about the redox status of the tissue because ubiquinol functions as an antioxidant, while ubiquinone does not. In fact, ubiquinol is the first line of defense in response to an oxidative stress (11, 32). Ubiquinol may readily react with the tocopheroxyl radical and be oxidized, or it may react directly with peroxyl radicals (33). Nonetheless, ubiquinols are depleted prior to α-tocopherol. Tissues involved in detoxification, both the liver and the kidney, have extraordinarily high concentrations of ubiquinol, perhaps to protect them from radicals escaping from p450. In addition, these tissues have high concentrations of mitochondria, which could also account for their high coenzyme Q contents (8). Previously, human and rat tissues, including heart, kidney, and liver, have been reported to contain high concentrations of ubiquinones (34). In general, the reduced fraction (ubiquinol) was higher in human than rat, and higher still than the mouse data reported here (34). Ernster (35) has suggested that higher tissue concentrations of ubiquinone may be a result of a higher metabolic rate. The mice studied here are hairless, therefore, to maintain their body temperature, they might have a higher rate of metabolism.

In summary, the data presented here give provocative clues to the uptake and regulation of tissue lipophilic antioxidants. The method described will, therefore, be useful for analysis of tissue antioxidant concentrations in response to various oxidative stresses.

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