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# HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF COENZYME Q-RELATED COMPOUNDS AND ITS APPLICATION TO BIOLOGICAL MATERIALS

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## SUMMARY

A convenient and precise method for the separation and determination of coenzyme Q (CoQ)-related compounds (CoQ homologues, plastoquinone-9, ubichromenol-9, etc.) was developed using high-performance liquid chromatography (HPLC). All compounds tested were separated using a reversed-phase column with a suitable mobile phase and detected at a wavelength of 275 nm. CoQ extracts in plasma and erythrocytes were purified by thin-layer chromatography prior to HPLC analysis, but such purification was not necessary when determining CoQ in urine and tissues. Hydroquinone forms of CoQ existing in animal tissues were oxidized to the corresponding quinone forms with potassium hexacyanoferrate(III).

This HPLC method was applied satisfactorily to the determination of the contents of CoQ homologues in human and animal samples. $CoQ_{10}$ was the only homologue detected in human samples, and $CoQ_8$, $CoQ_9$ and $CoQ_{10}$ were native homologues of CoQ in rat tissues. Ubichromenol-9 and plastoquinone-9 were not detected in these samples.

## INTRODUCTION

It is well known that coenzyme Q (CoQ) is an essential component of the mitochondrial respiratory chain. It serves as a carrier of electrons from flavoprotein dehydrogenases to the cytochrome system and for concomitant proton translocation across the mitochondrial inner membrane to conserve chemical energy of reducing equivalents for ATP synthesis [1, 2].

In general, the demand of CoQ for the physiological function seems to be

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supplied by biosynthesis de novo in mitochondria and in part by the dietary intake of CoQ itself. However, in some papers it has been suggested that the coenzymatic activities of CoQ in mitochondria in patients with heart disease [3], essential hypertension [4] or periodontitis [5] are lower than those of normal subjects and that administration of $CoQ_{10}$ improves some signs and symptoms in such patients [6, 7]. $CoQ_{10}$ has generally been used for the treatment of mild congestive heart failure in Japan since 1974. However, it is still obscure how exogenous CoQ affects the biochemical functions and the metabolism of endogenous CoQ.

In order to investigate such CoQ problems, a sensitive and accurate method for the determination of CoQ-related compounds is required. For the determination of CoQ in biological materials, a modified Craven's method [8] and a differential ultraviolet-absorption method [9] have often been employed in the past. However, these methods cannot easily distinguish each homologue of CoQ and need a large amount of sample because of low sensitivity. More recently, many papers have described high-performance liquid chromatographic (HPLC) methods [10—20]. However, most of them were applied only to longer homologues of CoQ such as $CoQ_9$ and $CoQ_{10}$ and did not consider other CoQ-related compounds such as ubichromenols, CoQ catabolites and plastoquinones, which have been found in biological samples [21, 22].

In this paper, we describe a convenient and precise method using HPLC for the separation and determination of CoQ-related compounds, methods for the preparation of CoQ samples from various biological materials and application to human plasma (serum), urine, erythrocytes, and animal tissues.

## EXPERIMENTAL

### *Analytical conditions for HPLC*

*Apparatus.* The HPLC device was composed of either a Shimazu LC-3A pump (Shimazu Seisakusho, Kyoto, Japan) with a Rheodyne loop injector and a Shimazu SPD-2A UV detector, or a Jasco Twincle pump (Japan Spectroscopic, Tokyo, Japan) with a VL-6 injector and a Jasco Uvidec 100-III UV detector.

*Chromatographic conditions.* The analytical conditions for the determination of CoQ were as follows: stationary phase, Finepak SIL $C_{18-5}$ (5-$\mu$m particles; stainless-steel column, 250 × 4.6 mm I.D.; Japan Spectroscopic); mobile phase, methanol—*n*-hexane (75:25); flow rate, 1.0 ml/min; measuring wavelength, 275 nm; sensitivity, 0.02 a.u.f.s.; and chart speed, 0.5 cm/min. Aliquots of 10 $\mu$l of the samples were injected into the column.

*Calculations.* The contents of CoQ homologues ($CoQ_n$) in the samples in $\mu$g of $CoQ_n$ per ml or g of biological material were calculated as follows:

$$\text{Content} = \frac{A}{B} \cdot \frac{1}{F} \cdot W \cdot \frac{1}{C}$$

where $A$ and $B$ are the peak heights of $CoQ_n$ and the internal standard (I.S.) in the sample solution, respectively, $F$ is the peak height ratio of standard $CoQ_n$ equivalent by weight to the I.S., $W$ is the weight ($\mu$g) of I.S. added

to the biological material and $C$ is the volume (ml) or weight (g) of the biological material used.

*Preparation of samples for HPLC*

All processes for the preparation of the samples were carried out in the dark. $CoQ_9$ and $CoQ_{11}$ (10 μg/ml in ethanol) were used as the I.S. for human and rat samples, respectively.

*Plasma (serum).* An aliquot of 0.5 ml of plasma from heparinized venous blood (serum) was pipetted into a glass-stoppered centrifuge tube, then 0.5 μg of I.S., 2 ml of methanol and 5 ml of *n*-hexane were added in turn. The mixture was shaken vigorously reciprocally at a rate of 80 times per minute for 10 min and centrifuged at 500 *g* for 10 min. Subsequently, 4 ml of the *n*-hexane layer were collected and concentrated in vacuo at room temperature and then the residue was dissolved in a small amount of *n*-hexane. The whole solution obtained was spotted on a thin-layer chromatographic (TLC) plate with a layer of silica gel 60 $F_{254}$ (Art. 5715; Merck, Darmstadt, F.R.G.) and developed with benzene—acetone (99.5:0.5). CoQ on TLC ($R_F$ = 0.44) was detected with a Chromatoscanner (Type CS-910; Shimazu Seisakusho, Kyoto, Japan) at 275 nm and collected in a glass-stoppered centrifuge tube. Then, 4 ml of acetone were added to re-extract CoQ from the silica gel powder. After shaking and centrifuging as described above, 3.5 ml of the acetone extract were collected, evaporated in vacuo and dissolved in 30 μl of ethanol.

*Erythrocytes.* The erythrocyte fraction was isolated from heparinized venous blood by centrifugation at 650 *g* for 10 min and washed three times with 2 vols. of 0.9% saline. The top part of the blood cell layer containing leucocytes and platelets was removed with the supernatant. The washed erythrocytes were suspended in 0.9% saline to give a haematocrit value of 40—60%. A 1-ml volume of the erythrocyte suspension was placed in a 300-ml separating funnel, then 1 μg of I.S., 20 ml of distilled water, 2 ml of 10% (w/v) methanolic pyrogallol and 1 ml of 50% potassium hydroxide solution were mixed homogeneously. Then, 40 ml of methanol and 80 ml of *n*-hexane were added to extract CoQ and, after shaking vigorously, the *n*-hexane layer was collected and washed twice with 20 ml of distilled water. The *n*-hexane layer was dried with anhydrous sodium sulphate, evaporated in vacuo and the residue was dissolved in a small amount of *n*-hexane. The CoQ in the extract was purified by TLC as described above for plasma samples. The acetone extract from TLC was dissolved in 30 μl of ethanol.

*Urine.* A 20-ml volume of urine was placed in a 300-ml separating funnel, and 1 μg of I.S., 40 ml of methanol and 80 ml of *n*-hexane were added. The mixture was vigorously shaken and the *n*-hexane layer was collected. The *n*-hexane extract was washed twice with 20 ml of distilled water, dried with anhydrous sodium sulphate and evaporated to dryness in vacuo. The residue was dissolved in 50 μl of ethanol.

*Rat tissues.* Rat tissues were homogenized with distilled water using a Polytron homogenizer (Type PT 10/35; Kinematica, Switzerland) at a setting of 7 for 30 sec. The final volume of the homogenate was adjusted to contain about 1—2 μg of CoQ. An aliquot of 0.5 ml of the tissue homogenate was pipetted into a glass-stoppered centrifuge tube and then 1 μg of I.S., 2 ml

of ethanol and 5 ml of *n*-hexane were added. The solution was shaken vigorously reciprocally at a rate of 80 times per minute for 10 min and centrifuged at 500 *g* for 10 min. Then, 4 ml of the *n*-hexane layer were collected and concentrated in vacuo and the residue was dissolved in 50 μl of ethanol. An aliquot of 0.5 ml of 5% potassium hexacyanoferrate(III) solution was added to the ethanol solution and the mixture allowed to stand for 10 min at room temperature. Subsequently, CoQ was re-extracted with 2 ml of ethanol and 5 ml of *n*-hexane as described above, and dissolved in 40 μl of ethanol.

*Measurement of creatinine and haemoglobin contents*

The creatinine content in urine and the haemoglobin content in erythrocytes were determined by the Folin—Wu method [23] and the cyanmethaemoglobin method [24], respectively.

*Materials and chemicals*

*Healthy human subjects.* These were all in normal ranges for the following twenty clinical laboratory tests for serum: glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase, γ-glutamyltranspeptidase (γ-GTP), lactate dehydrogenase (LDH), creatine kinase (CPK), total protein, albumin, cholesterol, triglyceride, total bilirubin, glucose, blood urea nitrogen, uric acid, creatinine, Na, K, Cl, Ca and P.

*Rats.* Four-week-old male Wistar rats (specific pathogen-free, body weight 60—80 g) were purchased from Shizuoka Agricultural Cooperative for Laboratory Animals (Shizuoka, Japan). They were fed on MF diet (Oriental Yeast, Tokyo, Japan) for two weeks in advance and grown to about 150 g body weight. Rats were anaesthetized with diethyl ether, injected with 0.2 ml of heparin (1000 U/ml) into the femoral vein and then laparotomized. First, blood was drawn from each abdominal aorta and each liver was perfused with 20 ml of 0.9% saline from the hepatic portal vein to the inferior vena cava. Then each tissue was cut out.

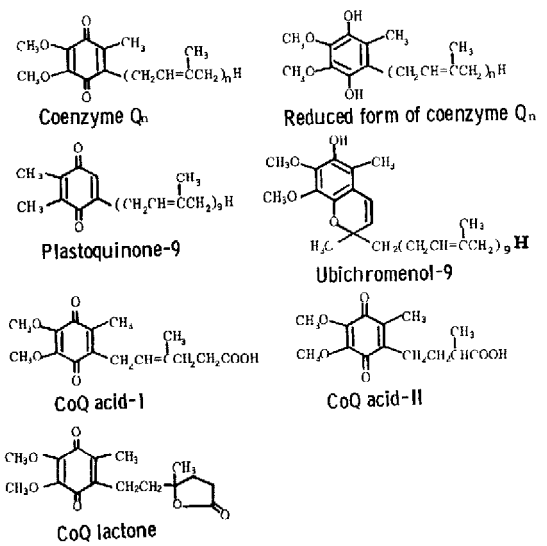


Fig 1. Structures of CoQ-related compounds.

*Chemicals*. CoQ homologues from $CoQ_5$ to $CoQ_{10}$, plastoquinone-9 and ubichromenol-9 were donated by Mr. S. Osono (Eisai, Tokyo, Japan). $CoQ_{11}$ was supplied by Dr. H. Fukawa (Nisshin Chemical Co., Saitama, Japan). *trans*-2,3-Dimethoxy-5-methyl-6-(5′-carboxy-3′-methyl-2′-pentenyl)-1,4-benzoquinone (CoQ acid-I) and *d*-2,3-dimethoxy-5-methyl-6-(3′-carboxybutyl)-1,4-benzoquinone (CoQ acid-II) were donated by Dr. H. Morimoto (Kobe-Gakuin University, Kobe, Japan). Hydroquinone derivatives of CoQ were prepared by the reduction of the corresponding CoQ homologues with sodium borohydride. 2,3-Dimethoxy-5-methyl-6-(5′-carboxy-3′-hydroxy-3′-methylpentyl)-1,4-benzoquinone lactone (CoQ lactone) was prepared by heating CoQ acid-I for 60 min at 70°C in 3 *M* hydrochloric acid [22]. The structures of the CoQ-related compounds are shown in Fig. 1.

All HPLC solvents were purchased from Wako (Osaka, Japan), and were of analytical-reagent grade for HPLC. Other reagents were of the best grade commercially available.

## RESULTS AND DISCUSSION

### *Separation of CoQ-related compounds by HPLC*

Table I gives the HPLC conditions for the separation of CoQ-related compounds with a Finepak SIL $C_{18-5}$ reversed-phase column. Generally, reversed-phase partition columns such as Nucleosil $C_{18}$ [16, 17], LiChrosorb Si 60

TABLE I

HPLC CONDITIONS FOR THE SEPARATION OF CoQ-RELATED COMPOUNDS AND RETENTION TIMES

| Mobile phase | Flow-rate (ml/min) | Compound | $t_R$ (min) |
|---|---|---|---|
| Methanol—*n*-hexane (90:10) | 1.0 | $CoQ_5$ | 5.7 |
| | | $CoQ_6$ | 6 9 |
| | | $CoQ_7$ | 8.6 |
| | | $CoQ_8$ | 10.8 |
| | | $CoQ_9$ | 14.1 |
| | | $CoQ_{10}$ | 18.6 |
| | | $CoQ_{11}$ | 25.0 |
| Methanol—*n*-hexane (75.25) | 1.0 | $CoQ_7$ | 6.2 |
| | | $CoQ_8$ | 7.3 |
| | | $CoQ_9$ | 8.6 |
| | | $CoQ_{10}$ | 10.4 |
| | | $CoQ_{11}$ | 12.8 |
| | | Ubichromenol-9 | 8.6 |
| | | Plastoquinone-9 | 11.0 |
| | | Retinyl palmitate | 7.8 |
| Methanol—water—acetic acid (65.35:0.5) | 0.8 | CoQ acid-I | 12.3 |
| | | CoQ acid-II | 9.0 |
| | | CoQ lactone | 10.6 |

[11, 19], LiChrosorb RP-18 [18], LiChrosorb RP-8 [19], μBondapak $C_{18}$ [10, 12] and Permaphase ODS [13—15] have been used to determine $CoQ_9$, $CoQ_{10}$ and other CoQ-related compounds. We also reported previously that Permaphase ODS, Zorbax ODS and Perkin Elmer HC-ODS SIL-X-I can be used to determine $CoQ_{10}$ in human plasma [25]. However, when comparing reversed-phase columns by means of the resolution value $[2(t_{R_2} - t_{R_1})/(W_1 + W_2)]$ for $CoQ_9$ and $CoQ_{10}$, the Finepak SIL $C_{18-5}$ column appeared to be better than others. In addition, all CoQ homologues from $CoQ_5$ to $CoQ_{11}$ could be separated successfully with the Finepak SIL $C_{18-5}$ column by varying the ratio of methanol to *n*-hexane in the mobile phase.

Fig. 2 shows the chromatogram of authentic $CoQ_5$ to $CoQ_{11}$ obtained with the Finepak SIL $C_{18-5}$ column. The retention times of the CoQ homologues decreased with decreasing ratio of methanol to *n*-hexane. With methanol—*n*-hexane (90:10), the CoQ homologues were clearly separated from each other but had long retention times (Fig. 2). When only $CoQ_7$ to $CoQ_{11}$ had to be separated, the most satisfactory result was achieved with methanol—*n*-hexane (75:25) as the mobile phase and a flow-rate of 1.0 ml/min. The retention times were 6.2 min for $CoQ_7$, 7.3 min for $CoQ_8$, 8.6 min for $CoQ_9$, 10.4 min for $CoQ_{10}$ and 12.8 min for $CoQ_{11}$. Under these conditions, the detection limit for $CoQ_{10}$ was 10 ng (signal-to-noise ratio = 5) and the peak heights of $CoQ_7$ to $CoQ_{11}$ increased linearly in the usual determination range up to 250 ng (2.5 μg/ml).

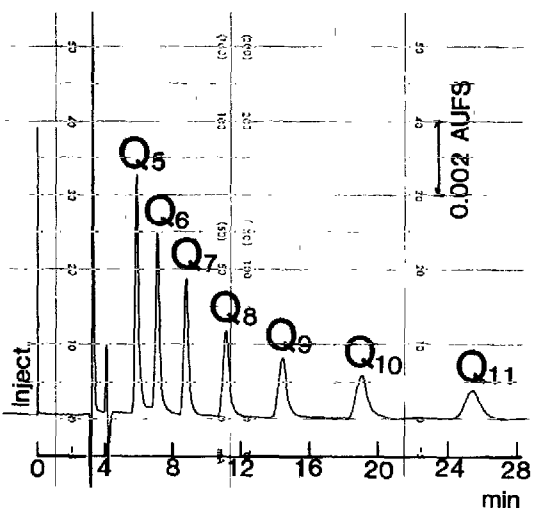


Fig. 2. Chromatograms of CoQ homologues. An aliquot of 5 μl of an ethanolic solution containing 50 ng of each CoQ homologue was injected. Conditions: mobile phase, methanol—*n*-hexane (90·10); stationary phase, Finepak SIL $C_{18-5}$; flow-rate, 1.0 ml/min; measuring wavelength, 275 nm; attenuation, *0.02 absorbance unit full scale* (a.u.f.s.).

With methanol—*n*-hexane (75:25), retinyl palmitate and plastoquinone-9 could also be separated from CoQ homologues. Katayama et al. [17] reported that a large amount of retinyl palmitate in liver extracts interfered with the determination of $CoQ_{10}$ under their HPLC conditions. Under our conditions,

however, it was eluted at 7.8 min, i.e. between $CoQ_8$ and $CoQ_9$ (Table I).

Plastoquinone-9 was eluted shortly after $CoQ_{10}$ but ubichromenol-9 had the same retention time as $CoQ_9$. However, in fact ubichromenol-9 is not detected in animal samples, as described later, and could be removed from the CoQ samples by the TLC purification method as described above.

CoQ catabolites of CoQ acid-I, CoQ acid-II and CoQ lactone were not detected under the conditions described above, but they were completely separated with methanol—water—acetic acid (65:35:0.5) as the mobile phase and eluted within 12.3 min. The retention times and the elution pattern of these CoQ catabolites are shown in Table I and Fig. 3, respectively.

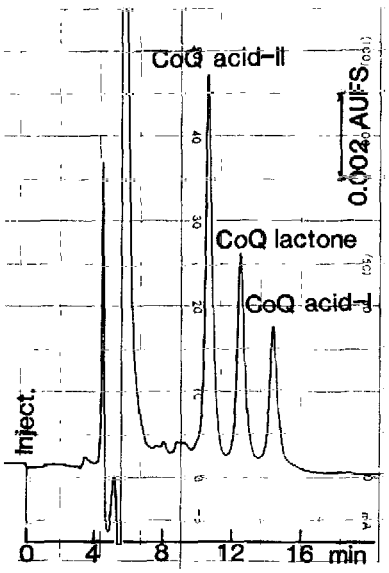


Fig. 3. Chromatograms of CoQ acid-I, CoQ acid-II and CoQ lactone. A mixture containing 1 μg each of CoQ acid-I and CoQ acid-II was heated at 70°C in 3 *M* hydrochloric acid for 60 min to partially lactonize CoQ acid-I, and extracted with 5 ml of benzene. The benzene layer was collected and concentrated and then the residue was dissolved in 50 μl of ethanol. An aliquot of 10 μl of the ethanolic solution was injected onto the HPLC column. Conditions: mobile phase, methanol—water—acetic acid (65:35:0.5); stationary phase, Finepak SIL $C_{18-5}$; flow-rate, 0.8 ml/min; measuring wavelength, 275 nm; attenuation, 0.02 a.u.f.s.

*Determination of CoQ in biological materials by HPLC*

The extraction of CoQ from biological materials has generally been performed with organic solvents such as *n*-hexane [16, 17, 25] directly or after alkaline saponification in the presence of pyrogallol [13, 14]. However, alkaline saponification has been suggested to cause partial isomerization of CoQ to ubichromenol and, if ethanolic potassium hydroxide is used, an exchange of methoxy groups of CoQ for ethoxy groups results, even under mild conditions [26]. In fact, alkaline saponification performed with 2.4% potassium hydroxide in the presence of 0.7% pyrogallol at 70°C for 10 min [13, 14] did not always give a satisfactory recovery rate when CoQ was added to biological materials. Therefore, a direct extraction method with *n*-hexane was chosen, as described under Experimental. A mixture of *n*-hexane with

methanol or ethanol was the most suitable solvent of those tested for the extraction of the homologues $CoQ_7$ to $CoQ_{11}$ at the same extraction rate. However, with lipid-rich samples such as serum, it was better to remove fat-soluble impurities from the CoQ extracts before HPLC analysis. Imabayashi et al. [15] employed enzymic hydrolysis with lipase to remove serum lipids. The purification of CoQ extracts from biological samples by TLC was superior to other pre-treatments prior to HPLC analysis. When developed on TLC plates with benzene—acetone (99.5:0.5), CoQ homologues of $CoQ_7$ to $CoQ_{11}$ showed about the same $R_F$ value of ca. 0.44. On the other hand, the $R_F$ values of ubichromenol-9 and plastoquinone-9 were 0.57 and 0.88, respectively. Therefore, such compounds were removed from the CoQ samples during the TLC purification. In erythrocytes, potassium hydroxide and pyrogallol were added to solubilize proteins prior to the *n*-hexane extraction. In some reports [13, 14], tocoquinone-9 has been used as an internal standard to correct the CoQ content. However, $CoQ_9$ or $CoQ_{11}$, if lacking in the samples, is more suitable than tocoquinone-9 because they behave similarly to the CoQ homologues in the process for the preparation of the CoQ extracts. In such a preparation process, in fact, the correlation coefficients between the peak height ratio and the concentration ratio of CoQ homologues to the I.S. ($CoQ_9$ or $CoQ_{11}$) were always more than 0.999. Therefore, $CoQ_9$ and $CoQ_{11}$ were used as a convenient I.S. when measuring CoQ contents in human samples and rat samples.

Fig. 4A and B show typical HPLC traces for CoQ homologues in human plasma. Plasma CoQ extracts purified by TLC gave clearer and more reliable HPLC patterns (Fig. 4B). Similar results were obtained for erythrocytes. The

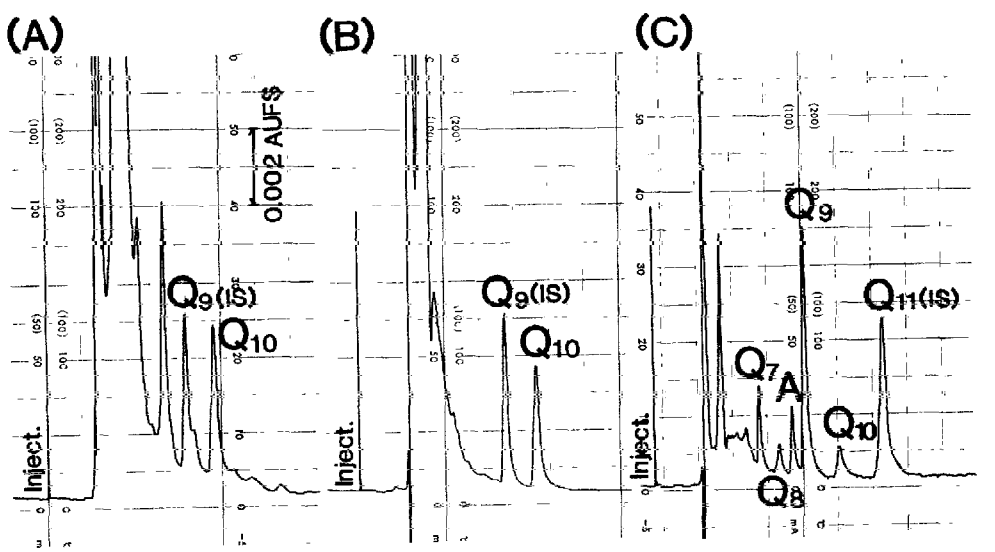


Fig. 4. HPLC of human plasma and rat liver samples (A) plasma sample before TLC purification; (B) plasma sample after TLC purification; (C) rat liver sample (peak A, retinyl palmitate) $CoQ_9$ and $CoQ_{11}$ were added to human plasma and rat liver samples, respectively, as described in the text. Conditions: mobile phase, methanol—*n*-hexane (75:25); stationary phase, Finepak SIL $C_{18-5}$; flow-rate, 1.0 ml/min; measuring wavelength, 275 nm; attenuation, 0.02 a.u.f.s.

TLC purification step could, however, be omitted for the determination of CoQ in urine samples.

The mean recovery of $CoQ_{10}$ added to human plasma was 103.6 ± 3.3% ($n$ = 9), which showed the good reproducibility of the method. The recoveries of $CoQ_{10}$ added to urine and erythrocytes were 102.2 ± 2.6% ($n$ = 6) and 105.6 ± 4.9% ($n$ = 6), respectively.

Mean plasma, serum, erythrocyte and urine levels of $CoQ_{10}$ in normal subjects are listed in Table II. These samples contained only $CoQ_{10}$. Therefore, $CoQ_9$ was used as a convenient internal standard when measuring $CoQ_{10}$ in these human samples. The mean levels of $CoQ_{10}$ in plasma and serum were 0.76 ± 0.15 and 0.77 ± 0.30 μg/ml, respectively, which are not significantly different from each other. Moreover, no significant difference was observed between mean plasma levels of $CoQ_{10}$ in 52 males and 26 females. Serum $CoQ_{10}$ of normal subjects was divided into five groups according to age, as shown in Table II, but no difference was observed in the serum $CoQ_{10}$ levels for each group. The $CoQ_{10}$ levels of normal human erythrocytes were less than 3% of those of plasma on the basis of volume. The $CoQ_{10}$ levels in random urine were in the range 0.020—0.085 μg/mg of creatinine. Daily urinary excretion levels of $CoQ_{10}$, therefore, were estimated to be 20—128 μg based on the daily urinary excretion levels of creatinine in normal subjects.

Direct extracts from tissue homogenates with $n$-hexane contained both oxidized and reduced forms of CoQ. It is necessary to oxidize the reduced form to the oxidized form in advance in order to determine the total CoQ content, i.e. the sum of the oxidized and reduced CoQ contents. The recoveries of $CoQ_9$, in reduced or oxidized form, added to rat tissue homogenates are

TABLE II

LEVELS OF $CoQ_{10}$ IN NORMAL HUMAN PLASMA, SERUM, ERYTHROCYTES AND URINE

| Subjects | Age* | $n$ | Levels of $CoQ_{10}$** |
|---|---|---|---|
| Plasma | | | μg/ml |
| Males | 21—34 (24) | 52 | 0.78 ± 0.15 |
| Females | 21—44 (23) | 26 | 0 73 ± 0.17 |
| Total | | 78 | 0.76 ± 0.15 |
| Serum | | | μg/ml |
| | 20—29 | 17 | 0.75 ± 0.16 |
| | 30—39 | 17 | 0.69 ± 0.23 |
| | 40—49 | 34 | 0.77 ± 0.31 |
| | 50—59 | 27 | 0.82 ± 0.25 |
| | 60 | 13 | 0.81 ± 0.19 |
| Total | | 108 | 0.77 ± 0.30 |
| Erythrocytes | | | μg/g of haemoglobin |
| Males | 23—30 (24) | 15 | 0.077 ± 0.022 |
| Urine | | | μg/mg of creatinine |
| Males | 21—43 (24) | 31 | 0.048 ± 0.016 |
| Females | 20—24 (21) | 24 | 0.050 ± 0.014 |
| Total | | 55 | 0.049 ± 0.015 |

*Average ages in parentheses
**Mean ± S D.

shown in Table III. The mean recovery was 100.8 ± 3.1% ($n$ = 9). The reduced form of $CoQ_9$, therefore, was oxidized to the corresponding quinone form completely by the addition of 0.5 ml of 5% potassium hexacyanoferrate(III) solution.

TABLE III

RECOVERIES OF $CoQ_9$, IN REDUCED OR OXIDIZED FORM, ADDED TO RAT LIVER HOMOGENATES

| $CoQ_9$ added | | $K_3Fe(CN)_6$ treatment | $CoQ_9$ found | | Recovery [§] (%) |
|---|---|---|---|---|---|
| Form | Amount ($A$) | | Before $CoQ_9$ addition ($B$) | After $CoQ_9$ addition ($C$) | |
| Reduced | 0.4* | — | 0 86* | 0.75* | 59.5 |
| | 0.8* | — | 0.86* | 1.41* | 84 9 |
| | 1.6* | — | 0.86* | 1.98* | 80.5 |
| Oxidized | 0.4* | + | 0 86* | 1.24* | 98 4 |
| | 0.8* | + | 0.86* | 1.64* | 98.8 |
| | 1.6* | + | 0 86* | 2.52* | 102.4 |
| Reduced | 0.3** | + | 0.54** | 0.83** | 98.8 |
| | 0.6** | + | 0.54** | 1.20** | 105.3 |
| | 1.2** | + | 0.54** | 1 66** | 95.4 |
| Reduced | 0.4*** | + | 0 95*** | 1.39*** | 103.0 |
| | 0.8*** | + | 0.95*** | 1.79*** | 102 3 |
| | 1.6*** | + | 0 95*** | 2.63*** | 103 1 |

*$\mu$g per 5.2 mg wet tissues.
**$\mu$g per 4.0 mg wet tissues
***$\mu$g per 5.8 mg wet tissues.
§ $\frac{C}{A+B} \cdot 100$

A typical chromatogram of a rat liver sample with $CoQ_{11}$ added as the internal standard is shown in Fig. 4C. CoQ homologues found in rat liver were $CoQ_7$, $CoQ_8$, $CoQ_9$ and $CoQ_{10}$ from their retention times and co-chromatography with the corresponding standard. As ubichromenol-9 was eluted at the same retention time as $CoQ_9$, we suspected that the peak $Q_9$ might contain ubichromenol-9. However, the peak disappeared on reduction of rat liver extract with sodium borohydride, and it appeared again on re-oxidation with potassium hexacyanoferrate(III). Further, from TLC and UV spectra, the peak $Q_9$ in Fig. 4C was confirmed to contain only $CoQ_9$. Under the given conditions, an appreciable amount of retinyl ester (probably the palmitate ester) was found in the rat liver samples tested and separated distinctly from CoQ homologues.

Table IV shows the results for CoQ contents in various rat tissues. The rat tissues contained four CoQ homologues, from $CoQ_7$ to $CoQ_{10}$. Among these, $CoQ_9$ predominated, its mean contents ($\mu$g/g wet weight, $\mu$g/ml) being 156.7 ± 3.3 for liver, 250.1 ± 7.1 for heart, 26.9 ± 0.8 for spleen and 0.47 ± 0.04 for plasma. $CoQ_7$ in liver and plasma was suspected not to be a native homologue but to have come from the diet during conditioning, as rats fed on a CoQ-depleted ration did not contain $CoQ_7$ in the liver or plasma.

TABLE IV

CoQ CONTENTS IN RAT TISSUES

| Tissue | CoQ content ($\mu$g/g wet weight for tissues, $\mu$g/ml for plasma)* | | | |
|---|---|---|---|---|
| | $CoQ_7$ | $CoQ_8$ | $CoQ_9$ | $CoQ_{10}$ |
| Liver | 29.7 ± 1.1 | 9.3 ± 0 8 | 156 7 ± 3.3 | 30.1 ± 0.7 |
| Heart | N.D.** | 9.2 ± 0.5 | 250.1 ± 7.1* | 27.0 ± 0.8 |
| Spleen | N.D. | 1.2 ± 0.1 | 26 9 ± 0.8 | 12.5 ± 0.4 |
| Plasma | 0 13 ± 0 02 | 0.08 ± 0.007 | 0.47 ± 0 04 | 0.17 ± 0.01 |

*Data are expressed as mean ± S.E. ($n$ = 6).
**N D. = not detectable.

In conclusion, CoQ-related compounds could be distinguished by HPLC using a Finepak SIL $C_{18-5}$ column. CoQ homologues in human samples and animal tissues could be determined more easily and routinely by HPLC. We have reported earlier [27] that the HPLC conditions described in this paper are applicable to content tests for pharmaceutical $CoQ_{10}$ preparations in Japan.

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