DETERMINATION OF UBIQUINONE AND TOCOPHEROL IN HEART, LIVER AND MUSCLE TISSUES OF FRESH WATER FISH, ROHU (LABEO ROHITA, HAMILTON)

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[The liver, heart and muscle tissues of rohu, (*Labeo rohita*, Hamilton) were examined for their ubiquinone (UQ)and tocopherol contents. These three tissues contained respectively 11.60, 3.94 and 0.19 mg. of ubiquinone and 10.16, 5.32 and 3.58 mg of tocopherol per 100 g. The 4 percent (V/V) of diethyl ether (EE) in light petroleum ether (PE) fractions of all three tissues on paper chromatographic separation gave spots having the same R_F value as standard ubiquinone-50 (UQ_{10}). Both the 4 per cent and 6 per cent (V/V) diethyl ether in light petroleum ether fraction of liver, heart and muscle tissues gave a single spot with the same R_F value as α - tocopherol.]

Introduction:

Ubiquinone is a naturally occurring lipid-soluble quinone of considerable biological importance. Lowe, Morton and Veron (Lowe *et al.*, 1957) and Heaton, Lowe and Morton (Heaton *et al.*, 1956) have examined respectively kidneys of many species and yeast unsaponifiable matter for ubiquinone contents. Later on a wide variety of material including several tissues were investigated for ubiqinone (Morton *et al.*, 1957, Mervyn and Morton, 1958; Mervyn and Morton, 1959; Lester and Crane, 1959, Diplock *et al.*, 1960) Ramasarma(1961) has reviewed the distribution of homologues in animals, plants and micro - organisms and concluded that CoQ10 is the predominant form in animal tissues.

Pennock, Morton and Lawson (1959, 1960) examined the fish tissues of marine and fresh water fish for quinones and related compounds. Recently Nazir and Magar (1964) examined the liver, heart and muscle tissues of shark (marine fish) for ubiquinone and tocopherol contents.

The physiological role of α-tocopherol is well known (Evans and Bishop (1922); Sure(1924); Devlin and Mattill 1942). Harris, Quaife and Swanson (1959), working with haddock reported α -tocopherol to constitute 90 per cent of the total tocopherol. Robeson and Baxter (1943) reported a natural antioxidant (α -tocopherol) from shark liver oil Einset, Olcott and Stansly (1957) have reported the tocopherol contents of various fish oils. Jansen and Kringstad (1942) observed that salmon and mackerel contained poor amount of tocopherol, while sardines contained an appreciable amount of tocopherol. Harris, Quaife and Swanson (1959) examined the tocopherol contents of fresh material and extracted lipids of haddock.

Practically no data is available on the presence of these compounds in fresh water fish, Rohu (*Labeo rohita*, Hamilton). The purpose of the present investigation was to study their occurrence in the tissues of fresh water fish, Rohu, and to find the particular homologues of ubiquinone and tocopherol present.

Materials and methods:

The fish were caught by gill net at Bandra Tank and brought to the laboratory.

The liver, heart and muscle tissues were removed as quickly as possible and about 200 g. of liver and muscle tissues and 25 g. of heart tissues were utilized for the experiment.

Saponification and Extraction– Saponification was carried out by a slight modification of the method used by Crane, Lester, Widmer and Hatefi(Crane *et al.*, 1959) and Lester and Crane (1959). Minced tissue was added to 300 ml. of 10 per cent (W/V) ethanolic KOH in which 10 g. of pyrogallol was previously dissolved and the whole mixture was refluxed for 30 minutes. After cooling the mixture was extracted thrice with isooctane (2, 2, 4 - trimethyl pentane) and made to known volume. Small aliquots from this solution were used for the spectra of ubiqninone and estimation of tocopherol.

After saponification the recoveries of standard ubiquinone and tocopherol were respectively 90-91.3 per cent and 97.3 per cent.

Removal of sterols– The iso - octane volume was reduced to about 25 ml. and the solution kept at 5°C overnight. The precipitate formed, mainly of sterols, was filtered off and washed with ice cold iso-octane and solutions were made to volume. The ubiquinone spectrum and estimation of tocopherol were repeated once more at this stage.

Column chromatography – In order to remove the interfering impurities and to separate the ubiquinone and tocophorol, column chromatography of the extracts was carried out by a slight modification of the method suggested by Mervyn and Morton (1959) and Jayaraman and Ramasarma (1961). Deactivated alumina (Merck, Brockman Grade III, deactivated with 4 per cent H_2O) was used as the adsorbent. After removal of the sterol the iso-octane was evaporated off under vacuum and the residue was dissolved in 5 ml. of petroleum ether (40-60°C). The column was packed with deactivated alumina (10 g. for each 100 mg of unsaponifiable matter) and washed with petroleum ether (PE) and further in succession with 4 per cent, 6 percent, 10 per cent and 20 per cent (V/V) of diethyl ether (EE) in light petroleum ether (PE) as eluents. The ubiquinone was eluted in the 4 per cent (V/V) EE-PE fraction which also contained small amounts of tocopherol, the remainder of the latter being eluted in the 6 per cent (V/V) EE-PE fraction. All the fractions were examined for ubiquinone and tocopherol contents.

The percentage recovery obtained when standard samples of ubiquinone and tocopherol homologues subjected to column chromatography were 92—94 per cent and 92 per cent respectively.

Paper chromathgraphy– Reversed phase chromatography was employed to separate the homologues. Whatman No. 1 filter papers impregnated with 5 per cent (W/V) solution of silicone stop-cock grease in chloroform were used for identifying the homologues of ubiquinone. The ascending method was used to develop the chromatograms using ethanol:water (85:15) as the mobile phase. The papers were developed for 26 hours, and immersed in 0.2 per cent $KMnO_4$ solution and then immediately washed with hot distilled water to remove the unchanged $KMnO_4$. The UQ compounds

TABLE-I. UBIQUINONE (mg/100 g of wet tissue) CALCULATED FROM READINGS OBTAINED AFTER DIFFERENT TREATMENTS

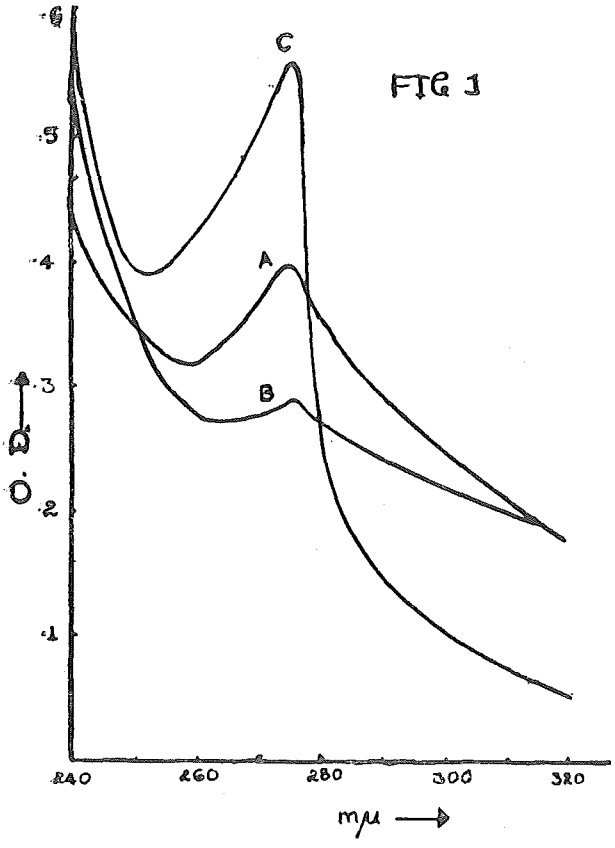
Tissue	Saponification	Removal of sterol	Column chromatography
Liver	*	*	11.60
Heart	*	4.53	3.94
Muscle	*	0.22	0.19

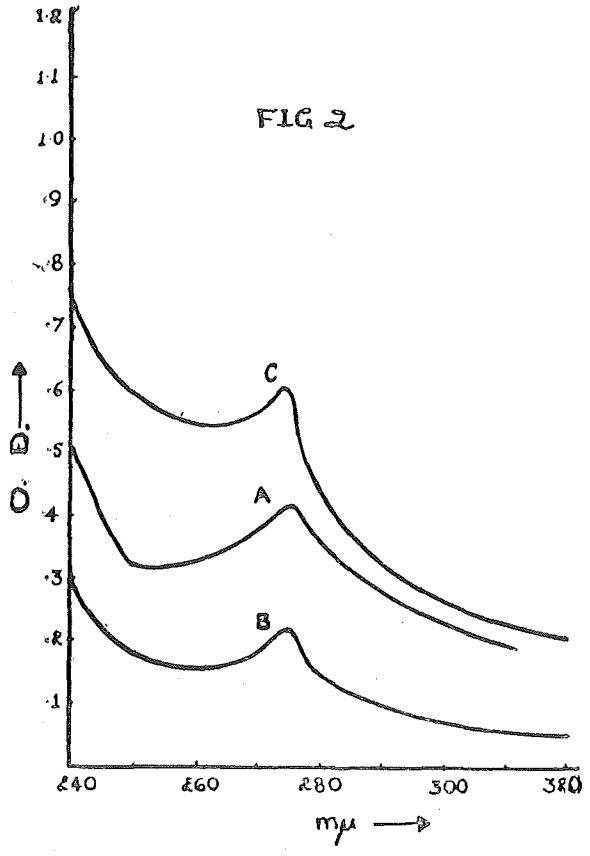
* Optical density was very high, so UQ content content could not be measured.

TABLE-II TOCOPHEROL CONTENTS (mg/100 g of wet tissue) CALCULATED FROM READINGS OBTAINED AFTER DIFFERENT TREATMENTS

Tissue	Saponification	Removal of sterol	Column chromatography
Liver	*	*	10.16
Heart	5.83	5.62	5.32
Muscle	4.14	4.08	3.58

* Optical density was very high, so tocopherol content could not be measured





ABSORPTION CURVES FOR UBIQUINONE IN ETHANOL

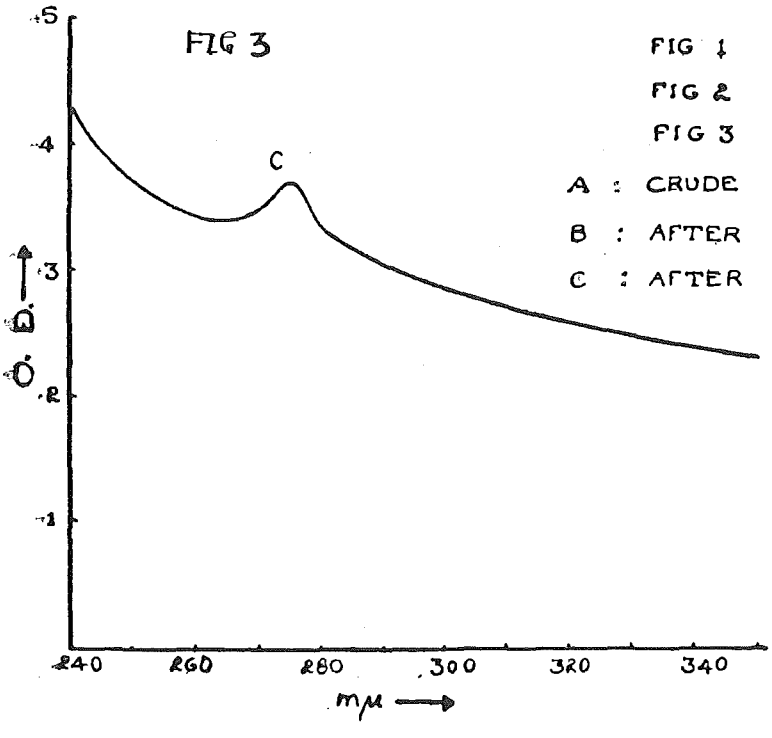


FIG 1 MUSCLE EXTRACTS
FIG 2 HEART EXTRACTS
FIG 3 LIVER EXTRACTS

A : CRUDE EXTRACT AFTER SAPONIFICATION
B : AFTER REMOVAL OF STEROL
C : AFTER COLUMN CHROMATOGRAPHY

appeared as prominent brown spots on a practically white background.

The particular form of the tocopherol was identified on an ascending chromatogram on a Whatman No.1 paper impregnated with 3 percent(V/V)liquid paraffin in petroleum ether (40-60°C) using an ethanol water (75:25) as the mobile phase. The chromatograms were sprayed with a 50 per cent (W/V) solution of phosphomolybdic acid in glacial acetic acid. The tocopherol appeared as blue spots on a yellowish green background.

Estimation of tocopherol- Estimation of tocopherol was carried out as suggested by Nazir and Magar. (1958)

Estimation of Ubiquinone (UQ)- Ultraviolet spectra of the ethanolic solutions of the standard and the samples for the estimation of UQ contents were taken on Beckman's DU spectrophotometer, both for exidized and reduced forms. The reduction was carried out with few grains of solid $NaBH_4$ (avoiding turbidity). The difference in the extinction coefficient ($\Delta E^{1\%}_{1cm.}$) at 275 mμ between the oxidized and reduced forms was used to calculate the amount of ubiquinone (UQ).

RESULTS AND DISCUSSION:

After saponification the absorption spectra of heart and muscle tissues showed a peak at 275 mμ, characteristic of ubiquinone and on reduction with solid $NaBH_4$ a decrease in absorption was noted. Liver tissue extracts did not show any characteristic peak at 275 mμ but an increase in readings with increase in wave length was observed. It might be due to other impurities present in these extracts and hence UQ content was not calculated at this stage.

The 4 per cent EE—PE fraction of liver tissue extracts obtained after column chromatogrophy showed a characteristic spectra of ubiquinone at 275 mμ, similar to the 4 per cent EE—PE fractions of heart and muscle tissues. Liver tissue extract showed decrease in absorption on reduction.

Even after removal of sterols, liver tissue extracts did not show characteristic peak at 275 mμ;whereas heart and muscle extracts gave similar absorption peak at 275 mμ. Similar findings were also observed by Nazir and Magar, (1964) Table I represents the ubiquinone contents of heart and muscle tissues at this stage. Due to other impurities the liver tissue extract gave a very high reading and hence CoQ content of liver was not calculated at this stage.

The content of ubiquinone in liver, heart and muscle tissues after column chromatography are representrd in Table I.

Pennock, Morton, Lawson and Laidman (1962) reported that the liver residue, heart and flesh of cod contained respectively 2.6, 49.0 and 0.24 μg CoQ_9 per g of wet tissue. Recently Nazir and Magar (1964) reported the CoQ contents of liver, heart and muscle tissues of shark as 10.83, 3.14 and 0.13 mg of CoQ per 100 g of wet tissue after column chromotography. The values obtained in the present investigation are quite different from those reported by previous workers and it might be due to the different species, different fishing season, habitat, feeding conditions, sex and maturity. On paper chromatographic analysis it was observed that all the three tissues (liver, heart and muscle) gave the spots having the same R_F value as standard UQ_{10}. The present findings confirm the earlier report of Pennock, Mortan, Lawson, and Laidman (Pennock *et al.* 1962). From this laboratory Nazir and Magar (1964) while working with shark tissues reported that heart and muscle tissues contained UQ_9 and liver tissue extract showed only UQ_6; whereas in the present study it was noticed that tissues of fresh water fish did

not contain any of the above reported lower homologues (UQ_6 and UQ_9) but only UQ_{10}.

The tocopherol contents of heart, muscle and liver tissues after different treatments are incorporated in table II. Liver extract gave very high readings after saponification as well as after removal of sterols and hence tocopherol content was not calculated after those treatments. After column chromatography tochpherol contents of liver, heart and muscle tissues were respectively 10.16, 5.32 and 3.58 mg. per 100 g of wet tissues. Pennock, Morton & Lawson, (1959) aud Pennock, Morton Lawson and Laidman (1962) reported that the liver, heart and flesh of cod contained respectively 24.6, 12.0, 3.3μg of α-tocopherol per g of wet tissues. Robeson and Baxter (1943) reported that shark liver oil contained 10 to 40 mg. of tocopherol per 100 g. Harris, Quaife and Swanson (1959) stated that haddock contained 0.39 mg of tocopherol per 100 g. of fresh material and 0.7 mg per g of extracted lipid. Jansen and Kringstad (1942) showed that sardines contained 4.5 mg of of tocopherol per 100 g. Nazir and Magar (1964)reported the tocohpherol contents of liver, heart and muscle tissues after column chromatography respectively 9.8, 4 88 and 2.94 mg per 100 g of fresh tissue. The values in the present study are slightly higher than those reported by Nazir and Magar (1964) and Harris Quaife and Swanson (1959) and lower than those reported by Pennock *et al* (1959, 1962) and Robeson and Baxter (1943). It is observed that every worker has got different values for tocopherol contents from different fish. The reason might be due to different species, maturity, sex, age, different dietary source of tocopherol and different habitat.

The slightly high values in the present study may be accounted for good dietary source of tocopherol.

Both 4 per cent EE-PE and 6 percent EE-PE fractions of liver, heart and muscle tissues when spotted on paper gave only one spot having the same R_F values as α-tocopherol. The presence of only α - tocopherol was reported from tissues of different fish by different workers. The present findings confirm these earlier findings that all the tissues examined contained only α - tocopherol.

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