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Determination of Ubiquinone and Tocopherol in Some Tissues of Shark (*Carcharias ellioti* Day)

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The presence of a quinone-like compound in the non-saponifiable portion of lipids in animal tissues was reported independently by two groups of workers, at Liverpool (Festenstien, Heaton, Lowe & Morton, 1955; Cain & Morton, 1955; Morton, 1958) and at Wisconsin, U.S.A. (Crane, Hatefi, Lester & Widmer, 1957; Lester, Crane & Hatefi, 1958; Lester, Hatefi, Widmer & Crane, 1959). However, it was soon recognized that several homologues of this quinone occurred in Nature; whereas in mammalian tissues the homologue having 10 isoprenoid units predominates, in plants and micro-organisms lower homologues having 6–9 isoprenoid groups have been reported (Lester & Crane, 1959a).

Seven different tocopherols have been found to occur in Nature (Blaxter, 1957), but α -tocopherol is the form most active biologically and is also the form in which tocopherol is preferentially stored in animal tissues. In haddock, the α -form constitutes over 90% of the total tocopherol (Harris, Quaife & Swanson, 1950).

Very little work has been reported on the ubiquinone and tocopherol contents of fish tissues and practically no data are available on the presence of these compounds in shark, although Robeson & Baxter (1943) reported a tocopherol content of 10–40 mg./100 g. of shark-liver oil. The present work was undertaken to determine the ubiquinone and tocopherol contents of the different tissues of shark and the particular homologue present.

EXPERIMENTAL

Saponification and extraction. The liver, heart and muscle tissues of freshly landed sharks (*Carcharias ellioti* Day) were used. Saponification was carried out by a slight modification of the method used by Crane, Lester, Widmer & Hatefi (1959) and Lester & Crane (1959b). About 200–250 g. of the minced tissue was added to 300 ml. of ethanolic 10% (w/v) KOH in which 10 g. of pyrogallol was dissolved, and the mixture was refluxed for 30 min. After cooling, the mixture was extracted thrice with iso-octane (2,2,4-trimethylpentane) and made to volume. This solution was used for the estimation of tocopherol and examined for the spectra of ubiquinone.

Removal of sterols. The volume of the 2,2,4-trimethylpentane extracts was then reduced to about 25 ml. and the solution kept at 5° overnight. A precipitate, which consisted mainly of sterols, was filtered off and washed with ice-cold 2,2,4-trimethylpentane; the solutions and washings were made to volume. The estimation of tocopherol and the spectrum of ubiquinone were carried out once more at this point.

Column chromatography. The method suggested by Mervyn & Morton (1959) and Jayaraman & Ramasarma (1961) was used with slight modifications. The 2,2,4-trimethylpentane was evaporated off under vacuum and the residue was dissolved in a few millilitres of light petroleum (b.p. 40–60°). A column was packed with alumina (Merck, Brockman grade, deactivated with 4% of water), 10 g. of adsorbent being used for each 100 mg. of unsaponifiable matter (Mervyn & Morton, 1959), and washed with light petroleum before use. After addition of the sample, the chromatogram was developed by using pure light petroleum, 4%, 6%, 10% and 20% (v/v) of diethyl ether in light

petroleum in succession as eluents. The eluates were examined for ubiquinone and tocopherol contents.

Paper chromatography. Whatman no. 1 filter paper was impregnated with a 5% (w/v) solution of Dow-Corning Silicone Stopcock Grease in CHCl_3 . Ethanol-water (17:3, v/v) was used as the mobile phase and the chromatograms were allowed to develop by the ascending method for 26 hr. A 0.2% KMnO_4 solution was used for detecting the ubiquinone spots (Lester & Ramasarma, 1959).

Whatman no. 1 filter paper impregnated with 3% (w/v) of liquid paraffin in light petroleum (b.p. 40–60°) was used for separating the tocopherol homologues. Chromatograms were developed for 26 hr. by the ascending method, with ethanol-water (3:1, v/v) as the mobile phase. Tocopherol was detected by spraying the chromatograms with a 50% (w/v) solution of phosphomolybdic acid in acetic acid, when blue spots appeared on a yellowish-green background.

Estimation of tocopherol. Tocopherol was determined as described by Nazir & Magar (1958).

Estimation of ubiquinone. The difference in the extinction coefficient at 275 $\text{m}\mu$ between the oxidized and reduced forms was taken as a measure of the amount of ubiquinone (Crane *et al.* 1959; Crane, 1959; Ramasarma, 1961).

RESULTS AND DISCUSSION

In the present experiments, about 90% of standard ubiquinone and 95–97% of standard tocopherol homologues were recovered after saponification.

The ultraviolet spectra of the crude extract of liver tissue after saponification did not show the characteristic spectra of ubiquinone. However, extracts of heart and muscle tissues did show a slight peak at 275 $\text{m}\mu$ with a decrease in absorption at this wavelength after reduction with sodium tetrahydroborate. The spectrum of ubiquinone was probably obscured by impurities in all three extracts and hence no measurements could be made at this stage (Table 1). The determination of the tocopherol content on the crude extracts of heart and muscle tissues gave values of 5.52 and 3.74 mg./100 g. of tissue respectively. The extract of liver tissue gave a very high reading, probably due to interference from vitamin A (Table 1).

After the precipitation of sterols the extracts of heart and muscle tissues gave ultraviolet spectra

recognizable as ubiquinone, and the readings at 275 $\text{m}\mu$ showed a decreased absorption after reduction with sodium tetrahydroborate (Table 1). The extract obtained from liver tissue did not show the characteristic spectra of ubiquinone, but exhibited decreasing values throughout. Tocopherol estimations at this stage gave results that were only slightly lower than the values obtained before precipitation of sterols, showing that the latter did not interfere markedly.

When the extracts, after removal of the sterols, were subjected to column chromatography, both ubiquinone and tocopherol were present in the 4% ether fraction, but experiments with standards showed that neither compound interfered greatly with the estimation of the other. Ubiquinone was found only in the 4% ether fraction of both heart and muscle-tissue extracts; the fraction obtained from the liver-tissue extracts gave a comparatively flat peak at 275 $\text{m}\mu$, but did show a decrease in absorption on the addition of sodium tetrahydroborate (Table 1). Tocopherol was found in both the 4% and the 6% ether fractions (Table 1). The values obtained for the ubiquinone and tocopherol contents of heart and muscle tissues after column chromatography are somewhat lower than those obtained from readings taken after precipitation of sterols, probably owing to removal of interfering substances as well as a slight loss of these compounds during chromatography. However, column chromatography appeared to be essential for the determination of these compounds in liver tissues.

Data on the ubiquinone and tocopherol contents of fish tissues are not very readily available. Lester & Crane (1959*a*) noted that the skeletal muscle of walleyed pike contained 0.009 μmole of ubiquinone-45/g. Pennock, Morton & Lawson (1959) stated that the liver, heart and flesh of cod contained respectively 2.76, 48.6 and 0.235 $\mu\text{g.}$ of ubiquinone/g. of wet tissue, though they did not determine the particular homologue present. They also showed that tinned cod liver (oil and residues saponified together), when examined after purification, contained 9.8 $\mu\text{g.}$ of wet liver tissue.

Einset, Olcott & Stansby (1957) have reported the tocopherol content of various fish oils as

Table 1. *Ubiquinone and tocopherol contents of the liver, heart and muscle tissues of shark*

Each value is the average of four determinations. 'Very high' indicates values much greater than those obtained by column chromatography in each case.

Tissue	Ubiquinone (mg./100 g. of tissue) calc. from readings obtained after			Tocopherol (mg./100 g. of tissue) calc. from readings obtained after		
	Saponification	Precipitation of sterols	Column chromatography	Saponification	Precipitation of sterols	Column chromatography
Liver	Very high	Very high	10.83	Very high	Very high	9.81
Heart	Very high	3.85	3.14	5.52	5.38	4.88
Muscle	Very high	0.17	0.13	3.74	3.56	2.94

varying from 4 mg./100 g. for sardine oil to 62.8 mg./100 g. for sablefish oil, and Robeson & Baxter (1943) reported a tocopherol content of 10–40 mg./100 g. of shark-liver oil. Pennock *et al.* (1959) observed that the liver, heart and flesh of cod contained respectively 24.6, 12 and 3.3 μ g. of α -tocopherol/g. of wet tissue. Harris *et al.* (1950) stated that haddock contained 0.39 mg. of tocopherol/100 g. of fresh material and 0.7 mg./g. of extracted lipid. Jansen & Kringstad (1942) (cf. Harris *et al.* 1950) showed that sardines contained 4.5 mg. of tocopherol/100 g. (0.45 mg./g. of fat). However, according to Jansen & Kringstad (1942) (cf. Harris *et al.* 1950), other fish are poorer in tocopherol content, e.g. salmon and mackerel contained 1.6–1.8 mg./100 g. and herrings 0.5 mg./100 g.

To determine which particular homologue of ubiquinone and tocopherol was present in the tissues of shark, paper-chromatographic separation was employed. The 4% ether fractions of heart and muscle-tissue extracts gave spots having the same R_f as standard ubiquinone-45. Ramasarma (1961) has tabulated the distribution of ubiquinone homologues in animals, plants and micro-organisms and concluded that ubiquinone-50 is the predominant form in animal tissues. However, in walleyed-pike muscle, the ubiquinone-45 homologue was present (Lester & Crane, 1959*a*). Surprisingly, the 4% ether fraction of the liver-tissue extracts consistently gave only one spot having the same R_f as ubiquinone-30. No data on the presence of this homologue in animal tissues have yet been recorded.

Both the 4% and 6% ether fractions of liver, heart and muscle gave single spots having the same R_f as α -tocopherol. However, the liver fractions revealed the possible presence of some unknown compound that had not travelled at all on the paper. Moreover, streaking was observed for these fractions. Pennock *et al.* (1959) reported that only α -tocopherol was found in the liver, heart and flesh tissues of cod, and Harris *et al.* (1950) stated that α -tocopherol constituted over 90% of the total tocopherol present in haddock.

SUMMARY

1. The liver, heart and muscle tissues of the shark (*Carcharias ellioti* Day) contained 10.83, 3.14

and 0.13 mg. of ubiquinone and 9.81, 4.88 and 2.94 mg. of tocopherol/100 g. of tissue.

2. Paper chromatography of ubiquinone consistently gave a spot having the same R_f as standard ubiquinone-30 for the fraction obtained from liver tissue, whereas the extracts of heart and muscle gave spots having the same R_f as standard ubiquinone-45.

3. Only α -tocopherol was detected in these three tissue extracts.

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