ON THE BIOSYNTHESIS OF UBICHROMENOL

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The natural occurrence of ubichromenol, a cyclic isomer of coenzyme Q (ubiquinone) was questioned by Draper and Csallany (1960), while supporting evidence was reported by Hemming, Laidman, Morton and Pennock (1961). We present here data on incorporation of radioactive mevalonic acid into ubichromenol of rat liver and kidney representing its biosynthesis and thereby confirming its natural origin.

Mevalonic acid-2-C^{14} (Radiochemical Centre, Amersham, England; 25 μC./mg.) was given orally to eight rats (5 μC. each) and after 4 hr. the rats were killed under ether anaesthesia and livers (73 g.) and kidneys (15.8 g.) were removed. The minced tissues were saponified for 30 min. under reflux in ethanol (10 ml./g. tissue) with sodium hydroxide (0.4 g./g. tissue) in the presence of pyrogallol (0.1 g./g. tissue). The unsaponifiable lipids were extracted with light petroleum (40-60°). The concentrated extracts were chromatographed on columns of alumina (Merck, chromatography grade, deactivated by adding 0.8 ml. water/20 g.) using successively the following eluents: light petroleum (200 ml.), 5% ether - light petroleum (100 ml.), 10% ether - light petroleum (100 ml.) and 20% ether - light petroleum (100 ml.). Fractions of 10 ml. were collected and assayed for radioactivity.

Under these conditions, hydrocarbons were eluted in light petroleum, coenzyme Q in 5% ether, ubichromenol in 10% ether and sterols in 20% ether. Four peaks of radioactivity, one each in the above fractions, were obtained. Owing to the interference by traces of sterol, the two peaks in 5% and 10% ether fractions were not distinct in the first column pattern. Therefore, the two fractions were pooled and rechromatographed. There were two prominent radioactive peaks in 5% and 10% ether fractions which coincided with the contents of coenzyme Q and ubichromenol, respectively (Fig. 1). The fractions corresponding to coenzyme Q and ubichromenol were pooled and subjected to repeated crystallization in ethanol (-20^{o}). The crystals, in both cases, retained radioactivity even after four crystallizations. The specific activity remained constant in the last three crystallizations showing thereby that coenzyme Q and ubichromenol of rat liver and kidney were labelled (Table 1).

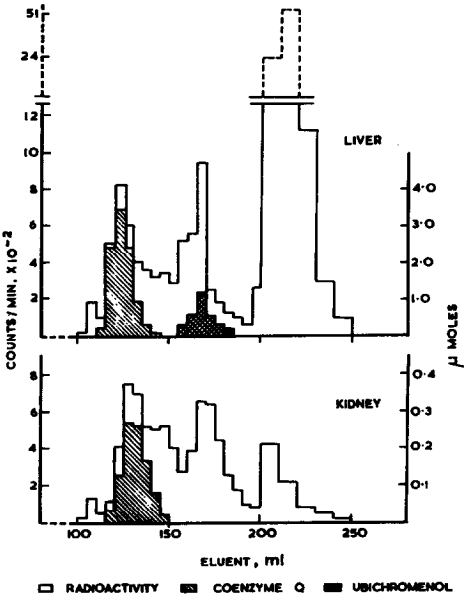

Fig. 1. Column chromatography of the unsaponifiable lipids of liver and kidney of rats treated with mevalonic acid-2-C^{14}.

Table 1

	Liver		Kidney	
	Q	UC	Q	UC
		μmoles		
Total	9.84	3.42	0.92	0.12
		Counts/min./μmole		
Original sample*	266	960	3,243	22,400
After adding the carrier**	-	-	1,920	2,200
Crystallizations:				
first	260	840	1,550	863
second	280	512	1,615	278
third	236	505	1,550	250
fourth	264	550	1,640	248
Average***	260	522	2,716	5,450
		Counts/min.		
Total	2,558	1,785	2,499	654

Q = Coenzyme Q; UC = Ubichromenol

* The ubichromenol samples used for crystallizations were treated with digitonin to remove traces of sterols.

** Liver samples were crystallized without addition of carriers since they had large quantities of the compounds. For kidney samples, 0.64 μmole of coenzyme Q_{10} and 1.16 μmole ubichromenol$_{10}$ were added to appropriate fractions.

*** The average specific activities were calculated from data on the last three crystallizations and corrected for the carriers added in the case of kidney samples.

Two striking features of the incorporation data deserve mention. The specific activity of ubichromenol is about twice that of coenzyme Q in both liver and kidney. The specific

activities of both coenzyme Q and ubichromenol in kidney are about ten times those of liver samples.

The fractions eluted with 5% and 10% ether from the first column were rechromatographed on columns of alumina (Merck, chromatography grade, deactivated by adding 0.4 ml. water/10 g.) using successively the following eluents: light petroleum (100 ml.), 5% ether - light petroleum (50 ml.), 10% ether - light petroleum (50 ml.) and 20% ether - light petroleum (50 ml.). 5 ml. fractions were collected. Radioactivity was measured in each fraction and coenzyme Q and ubichromenol were estimated spectrophotometrically in appropriate fractions.

In initial experiments using coenzyme Q_{10}-C^{14}, it was established that saponification and column chromatography on deactivated alumina used in the isolation procedure do not produce any significant amounts of ubichromenol from coenzyme Q (Joshi, Jayaraman and Ramasarma, 1963). It was also shown that coenzyme Q_{10}-C^{14} embedded in rat liver (after oral administration) was not converted to ubichromenol (Jayaraman, Joshi and Ramasarma, 1963). Further, in this and other experiments, it was shown that mevalonic acid-2-C^{14} was incorporated in ubichromenol with higher specific activity than in coenzyme Q. Therefore, it is concluded that ubichromenol is not formed from coenzyme Q, either during isolation or in the animal body, and that it is biosynthesized in the rat.

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