THE INCORPORATION OF THE METHYL GROUP OF [METHYL-^{14}C] METHIONINE INTO UBIQUINONE IN THE RAT

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The recently discovered lipid, ubiquinone (Coenzyme Q) was identified as a 3,4-dimethoxy 2,5-toluquinone derivative with a vitamin K_2-like isoprenoid side chain of 50 carbon atoms (Morton, Wilson, Lowe and Leat, 1958; Lester, Crane and Hatefi, 1958). This and other homologues have been found widely distributed in a variety of tissues (Gloor et al., 1958; Lester and Crane, 1959). It is localised in mitochondria (Lester et al. loc. cit; Hemming, Pennock and Morton, 1958) and is connected with the electron transport system (Crane, Hatefi, Lester and Widmer, 1957). It is however the first biologically active compound containing a methoxyl group to be isolated from animal tissues. Previously, this group was encountered only among the detoxication products of catechol derivatives, which are excreted in the urine following methylation (Axelrod, Senoh and Witkop, 1958; Axelrod, Rao and Goldzieher, 1960; De Eds, Booth and Jones, 1957; Booth et al., 1959). The immediate donor for O-methylation has been shown to be S-adenosyl methionine just as for N- or S- methylation.

It is of interest to know if an O-methyl transferase also participates in the biosynthesis of ubiquinone. Consequently [methyl-^{14}C] methionine was administered orally to rats to determine if the methoxyl groups of ubiquinone become labelled. Parallel experiments were carried out in vitro by incubating everted intestinal sacs of the rat with some of the labelled substance.

Experimental

In experiments I and II, [methyl-^{14}C] methionine (44μc./mg.) was administered orally to 2 groups of rats. Each rat received two doses of 5μc. each at 24 hr. intervals. The animals were killed 24 hr. after the last dose and the ubiquinone isolated from the livers and intestines by the method of Mervyn and Morton (1959) and estimated spectrophotometrically (Lawson et al., 1961). The ubiquinone-45 was crystallised to constant specific activity with the aid of carrier. The sterols were also isolated from the unsaponifiable matter as digitonides, which were then decomposed to liberate the free sterols.

In experiments III and IV, intestinal sacs were prepared from 6 female rats (Wilson and Wiseman, 1954) and incubated with the [methyl-^{14}C] methionine in bicarbonate buffer pH 7.4 under O_2/CO_2 (95/5). At the end of the incubation period the intact tissue and free mucosal cells were separated from the medium and the ubiquinone and sterols isolated as before.

Since the methyl group of methionine can be oxidised to CO_2 (Mackenzie et al., 1950) and reincorporated to some extent into β-hydroxy β-methyl glutaconic acid (Lynen et al., 1959 and Camillo-Campbell et al., 1959) a precursor of isoprenoid units, it was necessary to know the degree of labelling likely to be introduced into the side chain of ubiquinone. It has been shown that the specific activity of ^{14}C-ubiquinone formed from 2-^{14}C-acetate is generally less than twice that of the ^{14}C-cholesterol from the same tissue (Lawson et al., 1961). Hence the specific activities of the 3β-hydroxy sterols were also determined to give a measure of the radioactivity likely to appear in the side chain of ubiquinone.

Results

In Table 1 the values are given for the specific activities of the ubiquinone fractions (uncorrected for carrier ubiquinone)

during purification. Since the values become constant after the second crystallisation, it can be concluded that the ubiquinone has definitely become labelled.

Table 1

Specific activities of ubiquinone-45 during purification

Fraction	LIVER		INTESTINE	
	Weight mg.	Specific activity cpm./mg.	Weight mg.	Specific activity cpm./mg.
From column	12.7	228	9.5	127
1st crystals	8.8	174	3.1	107
2nd "	6.8	162	2.3	107
3rd "	2.5	168	-	-

The results for the specific activities of the endogenous ubiquinones and sterols for both the *in vivo* and *in vitro* experiments are listed in Table 2. The specific activities of the values for the

Table 2.

Specific activities of endogenous ^{14}C-labelled ubiquinone and 3β-OH sterols from the liver and intestine of the rat dosed orally with [methyl-^{14}C] methionine and from intestinal sacs incubated with the latter.

Expt.	Number and sex of rats	[methyl-^{14}C] methionine added μc	Time examined hr	Tissue	Specific Activity	
					Uq-45 cpm/mg	3β-OH sterols cpm/mg
In vivo						
I	3 F	30	24 hr after dosing	Liver	860	13
				Intestine	3700	11
II	3 M	15.5		Liver	431	6
				Intestine	2330	14
In vitro			Incubation period			
III	6 F	20.8	3 hr	Everted Intestinal Sacs	292	4
IV	6 F	15.5	8 hr		743	5

ubiquinone fractions are invariably much greater (70-300 times) than the corresponding ones for the 3β-OH sterols. These indicate that the substituted aromatic moiety of the ubiquinone molecule becomes labelled presumably in the methoxyl groups to a larger extent than the polyisoprenoid side chain which at best would only be twice that of the sterols. Conclusive proof that the methoxyl groups were labelled was obtained later by subjecting the bulked crystals (6.7 mg. 700 counts/min.) from the livers and intestines to a Zeisel degradation. The methyl iodide liberated was distilled into an alcoholic solution of trimethylamine. The tetramethyl ammonium iodide formed contained 430 cpm. compared to 180 cpm. which remained in the reaction mixture.

Discussion

The fact that the radioactive label is incorporated into the methoxyl group(s) of ubiquinone *in vitro* as well as *in vivo* implies that the animal methyl-transferase rather than those of intestinal microflora is concerned in the methylation. Furthermore, most bacteria are known to make the lower homologue, ubiquinone-40, rather than ubiquinone-45 isolated in these experiments (Pandya, Bishop and King, 1961; Lester and Crane, 1959).

Since no evidence has yet been obtained to suggest that O-methylation is reversible, it may be assumed for the present that the animal can utilise for ubiquinone synthesis an aromatic precursor which is not fully methylated. The stage at which the methylation occurs is not clear. From the work of Stoffel and Martius (1960) it seems that 3,4-dimethoxy 2,4-toluquinone is a precursor of ubiquinone in the same way as 2-methyl-1,4-naphthoquinone is used for vitamin K_2 synthesis. Thus methylation apparently occurs prior to the coupling of the isoprenoid side-chain. Indeed, the finding of about one-quarter of the total ^{14}C-label incorporated, distributed in other parts of the molecule than in the methoxyl groups, tends to confirm that the methyl label was

introduced during de novo synthesis of the ubiquinone rather than by turnover of only the methoxyl groups.

The failure of Rudney and Sugimura (1960) to find radioactivity in ubiquinone-45 or -50 following the administration of [methoxyl-^{14}C] ubiquinone-30 should not perhaps be interpreted as meaning that the nucleus of ubiquinone-30 cannot be used for ubiquinone-50 biosynthesis. The methoxyl label may be lost during the metabolism of the ring moiety before the latter is used for new ubiquinone synthesis.

Summary

The methyl group of [methyl-^{14}C] methionine is incorporated in small amounts into the ubiquinone and sterols of (a) rat liver and intestine in vivo and (b) everted intestinal sacs of the rat in vitro. The specific activity of the ubiquinone is 70-300 times greater than that of the sterols. Most of the radioactive label in the former is present in the methoxyl groups.

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