ON THE BIOSYNTHESIS OF COENZYME Q_9 IN THE RAT*

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Coenzyme Q_{10} has been isolated from a variety of mammalian sources including beef (Crane et al., 1957; Linn et al., 1959), human (Linn et al., 1959), horse (Bouman et al., 1958), and pig (Fahmy et al., 1958), and has been identified by spectra and paper chromatograms in rat, mouse, chick, turkey and rabbit tissues (Linn et al., 1959). Lower homologues of coenzyme Q, including Q_9, have been identified in microbial sources (Lester, Crane and Hatefi, 1958). The finding of both coenzyme Q_9 and Q_{10} in rat and mouse tissues (Linn et al., 1959) raised the question of the origin of these two homologues in these species. Earlier studies in this laboratory (Dialameh and Olson, 1959) showed that acetate-1-C^{14} was incorporated into the coenzyme Q isolated from rat liver by chromatography on alumina and purified to constant specific radioactivity with carrier coenzyme Q_{10}. Similarly, Gloor and Wiss (1959) reported that mevalonic acid-2-C^{14} was incorporated into coenzyme Q isolated from rat liver by chromatography on alumina and crystallized to constant specific activity with carrier coenzyme Q_{10}. The inference drawn from these studies was that the native form of the coenzyme in rat liver, as in most mammalian tissues, is Q_{10}.

Since it was soon evident that chromatography on alumina was not able to separate coenzyme Q_9 from Q_{10}, the nature of the coenzyme Q in rat liver was

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reinvestigated. 2.0 kilograms of rat liver were extracted with ethanol:ether 3:1, the extract evaporated to damp dryness, and saponified with methanolic KOH. The nonsaponifiable fraction was extracted with petroleum ether, washed, dried, and chromatographed on Brockman's alumina grade III. The yellow coenzyme Q band was eluted with 4% ethyl ether:petroleum ether and rechromatographed to spectral purity, i.e. $\Delta\lambda \overset{\text{Ethanol}}{0\longrightarrow R}$ 275 mμ ($E^{1\%}_{1\ cm}$ = 157). At this stage reverse phase paper chromatograms, (Linn et al., 1959) revealed a mixture of approximately 80% coenzyme Q_9 and 20% coenzyme Q_{10}. This mixture was subjected to further chromatographic purification on polyethylene powder or paper impregnated with vaseline and the product was crystallized twice from methanol. 42 mg. of orange crystalline material were obtained which melted at 44-45°, $\lambda^{\text{Ethanol}}_{\text{max}}$ 275 mμ ($E^{1\%}_{1\ cm}$ = 188), $\lambda^{\text{Ethanol}}_{\text{min}}$ 236 mμ ($E^{1\%}_{1\ cm}$ = 30), $\Delta\lambda \overset{\text{Ethanol}}{0\longrightarrow R}$ 275 mμ ($E^{1\%}_{1\ cm}$ = 161) (Anal.; Calc. for $C_{54}H_{82}O_4$; C,81.56; H,10.39; CH_3O-,7.81; Found: C,81.43; H,10.24; CH_3O-,8.32). R_f (one spot) using silicone impregnated paper and propanol:water 4:1 was 0.38 (Lester and Ramasarma, 1959).

Metabolites found in animal tissues may arise from the diet, from microbiological synthesis in the gut, or from endogenous biosynthesis in the tissue itself. In order to clarify the origin of the coenzymes Q found in rat liver, a series of dietary and isotopic labelling experiments were carried out in albino rats. Acetate-1-C^{14} in doses of 50-500 μc (50 μM) was administered intraperitoneally to rats weighing 100-150 grams. The animals were sacrificed in 1.5 hours, and coenzymes Q, cholesterol and vitamin A isolated by chromatography on alumina (Dialameh and Olson, 1959). After additional chromatography on alumina to constant specific activity, the mixture of coenzymes Q was chromatographed on paper (Linn et al., 1959). The two spots for coenzyme Q_9 and Q_{10} were eluted separately. Cholesterol was purified via the digitonide. The radioactivity of all samples was determined by liquid scintillation counting. Vitamin A was found to be nonradioactive.

TABLE I

Incorporation of Radioactivity from Acetate-1-C^{14} into Hepatic Cholesterol and Coenzymes Q

Experiment	Radioactivity Administered as Acetate-1-C^{14} μc.	Radioactivity of Isolated Products		
		Cholesterol CPM/Mg.	Coenzyme Q_9 CPM/Mg.	Coenzyme Q_{10} CPM/Mg.
1	50	1,460	750	30
2	500	37,100	11,600	330

These data indicated that coenzyme Q_9 is the endogenous form of coenzyme Q in the rat, and that coenzyme Q_{10} is derived from fecal or dietary sources in this animal. In support of this hypothesis, it was found that hepatic coenzyme Q_9 was rapidly labeled with isotope from acetate-1-C^{14} in rats surgically deprived of their gastrointestinal tracts. In further support of this hypothesis are the dietary studies presented in Table II. Total coenzyme Q concentrations were determined on unsaponified extracts of liver chromatographed on alumina and the total $\Delta\lambda$ $\overset{\text{Ethanol}}{O\longrightarrow R}$ (KBH_4) measured. The ratio of Q_9:Q_{10} was determined by chromatography on paper.

TABLE II

Concentration of Coenzyme Q_9 and Q_{10} in Rat Liver under Various Dietary Conditions

Experiment	No. Rats	Diet	Supplement Daily	Coenzyme Q_9 μg/g.	Coenzyme Q_{10} μg/g.
1	6	Chow	-	91	19
2	6	Q-free*	-	95	17
3	6	Q-free*	2 mg CoQ_{10}	129	62

* The Q-free diet was a standard synthetic diet containing 18% casein and 6% lard (Olson et al., 1958).

Diets essentially coenzyme Q-free (Page et al., 1959) do not depress the

level of coenzyme Q in rat liver (Morton, 1958) or change the ratio of the two homologues. The source of coenzyme Q_{10} in the rat is probably microbiological since feces from rats on Q-free diets were found to contain 25 μg/gm of coenzyme Q equally divided between Q_9 and Q_{10}. The addition of coenzyme Q_{10} to the diet of the rat (2 mg/day) for a week increased the total coenzyme Q content of the liver and the proportion of Q_{10} present.

From these experiments it is concluded that coenzyme Q_9 is the endogenous form of the coenzyme in normal rat liver and that the coenzyme Q_{10} present is derived from microfloral or dietary sources. Degradative studies of isotopic coenzyme Q_9 from the rat (Olson, Dialameh and Bentley, 1960) suggest that the acetate-1-C^{14} is incorporated principally if not entirely in the isoprenoid side chain and possible precursors of the aromatic moiety of the molecule are under investigation. The large amounts of coenzyme Q_9 present in the livers of vitamin A-deficient rats (Gloor and Wiss, 1959), probably represents an increased synthesis of a normal constituent in this deficiency disease.

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