

Ubiquinone (coenzyme Q) in the germ-free rat

Several laboratories have shown that mevalonate^{1,2} or acetate^{2,3} are incorporated into the ubiquinone molecule in the intact rat. Mevalonate incorporation followed isoprenoid biosynthesis and was found in the side chain but not in the benzoquinone moiety of ubiquinone^{4,5}. The origin of the ring portion of the compound is not established and isotopic studies with phenylalanine are somewhat at variance^{4,6}. Attempts to alter tissue levels of ubiquinone by dietary means^{7,8} or by interfering with the intestinal flora⁹ have not succeeded in decreasing appreciably the amount of the compound in the body. Although these reports collectively strongly indicate that rat tissues have the ability to synthesize the ubiquinone molecule *in toto*, unequivocal proof could only be obtained by studying germ-free animals fed a chemically defined diet devoid of ubiquinone.

The diet for this experiment contained the following ingredients per 100 g: L-amino acid mixture¹⁰, 12.5; vitamin mixture*, 2; urea complex of linoleic acid**, 1.37; NaHCO₃, 0.88; sucrose, 83.25. Sterilization was effected by exposing the diet sealed in polyethylene bags to a dose of 3 million rads from a high-energy electron beam (Van de Graaf accelerator).

Chemical or microbiological analysis showed that the irradiation destroyed 45 % of thiamine, 20 % of riboflavin and 12 % of vitamin A. Growth of conventional weanling rats fed the irradiated diet was 2.3 g/day during a 21-day period. Analyses of the livers of these rats showed adequate stores of vitamin A and α -tocopherol.

A litter of 7 Lobund-strain rats, born in an isolator to a germ-free female, was used. The dam was fed a stock diet of mixed natural and purified ingredients. When the young were 10 days old the stock diet was replaced with the chemically defined diet. The rats were weaned when 21 days old and were continued on the diet *ad libitum*. At intervals, rats were removed from the isolator and the livers and hearts analyzed. The homogenized tissues were saponified in the dark for 15 min with ethanolic KOH in the presence of pyrogallol. The washed and dried hexane extracts were analyzed for ubiquinone, and in the case of liver, also for vitamin A and α -tocopherol. Ubiquinone was determined spectrophotometrically at 275 m μ in ethanol by the KBH₄-reduction procedure¹¹. Vitamin A was estimated with the Carr-Price reagent, and α -tocopherol with the Emmerie-Engel reagent after isolation by chromatography¹². Sterility of the isolator was checked periodically.

It is apparent from Table I that there was a marked increase in the total ubiquinone content of liver as the rats aged. Heart also accumulated ubiquinone. The range of concentrations in the livers of the germ-free rats 35 and 50 days of age is similar to that found in our conventional rats fed a purified diet containing 20 % vitamin-free casein and 4 % cottonseed oil. Rats number 6 and 7 should be considered separately inasmuch as these animals died spontaneously in the isolator. Autopsy revealed hemorrhages throughout the viscera suggestive of vitamin-K deficiency.

Green *et al.*¹³ have reported that Hormel's methyl linoleate contained approx. 5 μ g/g of ubiquinone. If true, our chemically defined diet would contain only 0.023 μ g ubiquinone/g of diet, and the ingestion of 250 g of diet over a 30-day period would contribute less than 6 μ g of ubiquinone to the body.

* Five times the amounts of all vitamins commonly added to purified diets in this laboratory were used.

** Hormel Institute, Austin, Minn. The complex contained 33 % methyl linoleate.

TABLE I
UBIQUINONE* CONTENT OF LIVER AND HEART OF GERM-FREE RATS

Rat No.	Age (days)	Sex	Body wt. (g)	Liver					Heart	
				Weight (g)	Ubiquinone		Vitamin A	Vitamin E	Ubiquinone	
					Total μ g	μ g/g	μ g/g	μ g/g	Total μ g	μ g/g
1	21	F	17**	0.52	75	144	21	—***	15	167
2	35	F	35	2.23	257	115	13	—	37	195
3	35	M	44	2.77	286	103	12	—	43	215
4	50	M	72	2.94	523	178	29	—	68	151
5	50	M	71	3.39	700	204	24	—	40	100
6	60	M	73	3.37	775	230	33	18	—	—
7	67	F	80	2.94	883	300	40	17	76	211

* Calculated as ubiquinone (45) or coenzyme Q_9 .

** This rat was considerably smaller than the others when weaned.

*** Blank spaces indicate the analysis was not performed.

Characterization of the ubiquinone in the livers of rats 6 and 7 by paper chromatography¹⁴ revealed only one zone corresponding to ubiquinone (45) or coenzyme Q_9 . The kidneys from these rats, however, when analyzed in the research laboratories of Merck Sharp and Dohme (through the courtesy of Dr. K. FOLKERS) were found to contain approximately 90% of ubiquinone (45) and 10% of ubiquinone (50). Both compounds can thus be synthesized in the rat body. It can be concluded that the complete biosynthesis of ubiquinone occurs in the tissues of the rat, and that no exogenous source of the preformed benzoquinone ring is necessary.

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