SOME OBSERVATIONS ON THE METABOLISM OF COENZYME Q AND UBICHROMENOL IN RAT TISSUES

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The increased concentrations of liver-coenzyme Q (ubiquinone) in rats deficient in vitamin A, which led to the discovery of this quinone, were observed as early as 1957 (1) but the cause remains still unknown. Protein deprivation in the diet of adult or weanling rats resulted in lowering the concentration of coenzyme Q in livers of rats (2). Similar changes were observed in the contents of ubichromenol, an isomer of coenzyme Q (1,2). It is possible that alteration in the rate of synthesis or the degradation of these compounds would result in the changes in their levels. The results of our investigations testing this possibility are summarized here and the details will be published elsewhere.

Incorporation of mevalonate-2-C^{14} into the isoprene side chain of these compounds is taken as the measure of their biosynthesis. The following experimental procedure was used: Albino rats (normal or vitamin A deficient or protein deficient) were dosed orally, or intraperitoneally where indicated, with the tracer and were killed under ether anaesthesia after specified time. The tissues--liver, kidney and intestine (small)--were saponified and the unsaponifiable lipids were chromatographed on

deactivated alumina columns. The fractions of 5% and 10% ethyl ether in petroleum ether, which contained coenzyme Q and ubichromenol, respectively, were further purified by thin layer chromatography on alumina and the specific radioactivities determined as described earlier (3,4).

Biosynthesis of coenzyme Q and ubichromenol in vitamin A deficient rats: Progressive with the deficiency of vitamin A in the rats, the liver-coenzyme Q concentration (5) and the amounts of mevalonate-2-C^{14} incorporated into coenzyme Q (6) increased. But in the earlier experiments of Gloor and Wiss (6) a time interval of 24 hr. after administration of the tracer was employed. Incorporation data of orally administered mevalonate-2-C^{14} (to normal rats) showed that the amount of radioactivity in liver-coenzyme Q increased with the increase in the time interval upto 8-12 hr. and then decreased (4). Therefore, earlier time intervals have to be used for rate studies. Using 2 hr., it was found that the rates of synthesis of coenzyme Q and ubichromenol in livers of vitamin A deficient rats (at plateau stage) showed considerable decreases compared to normals. At 72 hr. the radioactivity present in these compounds decreased in livers of normal rats but increased in vitamin A deficient rats (Table 1). Further, radioactive coenzyme Q_{10} and ubichromenol administered either orally or intracardially, in separate experiments, were deposited in livers of rats and the amount of the tracers in the livers of normal rats decreased with time but not in the deficient rats. These results point out that the observed higher concentrations of coenzyme Q and ubichromenol are probably due to their accumulation resulting from a block in their degradation. Such an explanation may well be true in the case of other conditions

Table 1

	Counts/min.			
	Coenzyme Q		Ubichromenol	
	2 hr.	72 hr.	2 hr.	72 hr.
Normal	1042	846	570	10
Vitamin A deficient	726	2690	226	463

Two rats per group were dosed orally 5 uC. mevalonate-2-C^{14} each. The values given are for two livers.

such as thyrotoxicosis (7,8) and cold exposure (9) where increased liver concentrations of coenzyme Q were reported. This possibilit is now under investigation.

Biosynthesis of coenzyme Q and ubichromenol under conditions of protein deprivation: The lowered levels of coenzyme Q and ubichromenol in livers of rats fed on 12% and 6% compared to 20% casein in the diet appear to be due to lowered rates of synthesis (Table 2). However, the incorporation of radioactivity into coenzyme Q and ubichromenol in livers of rats fed diet containing no casein was higher than that of rats fed on 6% casein. These results suggest that mild protein deficiency lowers the biosynthesis of these compounds and under conditions of severe protein deficiency their degradation might also be blocked in an effort to conserve coenzyme Q for oxidative reaction in the body.

Table 2

Casein in the diet (%)	Counts/min.	
	Coenzyme Q	Ubichromenol
20	854	588
12	293	269
6	240	427
0	546	571

Weanling rats were maintained on different diets for 30 days. Four rats per group were dosed orally 2 uC. mevalonate-2-C^{14} each and killed after 6 hr. The values given are for 4 livers

On the biosynthesis of ubichromenol in rat tissues: The biosynthesis of ubichromenol in rat tissues was demonstrated in this laboratory as tested by the incorporation of orally administered mevalonate-2-C^{14}. The specific radioactivity of ubichromenol was higher than that of coenzyme Q (3). This was contradicted by Green et al. (10) who carried out a similar experiment but used 24 hr. time interval and intraperitoneal administration of the tracer. Subsequently, we confirmed and extended our observations showing that the specific radioactivity of ubichromenol decreased and that of coenzyme Q increased with increase in the time interval upto 8 hr. in three tissues tested--liver, kidney and intestine (11). We also found that at 24 hr. the value for ubichromenol, although lower than coenzyme Q, was not as low as reported (10). On repeating the experiment by administering the tracer by intraperitoneal route and testing after 4 hr. we now find that ubichromenol of kidney and intestine was labelled but not of liver (Table 3). These

experiments suggest that kidney and intestine, but not liver, have the biosynthetic mechanism of ubichromenol, while the three tissues are capable of synthesizing coenzyme Q. Liver

Table 3

	Counts/min./umole			
	Coenzyme Q		Ubichromenol	
	Oral	i.p.	Oral	i.p.
Liver	1420	417	7980	41
Kidney	7450	1420	105300	317000
Intestine	10250	960	55400	4510

Four rats per group were dosed 5 uC. mevalonate-2-C^{14} each, as indicated and killed after 4 hr. i.p.-intraperitoneal.

ubichromenol is probably derived from intestine but not from intestinal bacteria since the latter do not possess ubichromenol. Also it cannot be argued that intestinal bacteria are responsible in some way for the formation of ubichromenol since this does not explain its synthesis in kidney after intraperitoneal administration of the tracer.

Site of synthesis of coenzyme Q in rat liver cell: It is now established that coenzyme Q is distributed in all the cell fractions of rat liver (12). Using an *in vitro* system it was claimed that the site of synthesis for the final step of condensation of the ring and the isoprene side chain is mitochondria (13). Employing essentially the same approach as used to find out that the microsomal fraction is the site of synthesis of cholesterol *in vivo*, we now find that the soluble supernatant is probably the site of synthesis of coenzyme Q. The specific radioactivities of coenzyme Q in the cell fractions

obtained from 10% sucrose homogenates of livers from rats dosed with mevalonate-2-C^{14} and killed after various time intervals were determined. At 1 hr. time interval these values for supernatant, microsomal, mitochondrial and nuclear fractions are 1060, 130, 397 and 294 (counts/min./umole), respectively. The value of supernatant-coenzyme Q decreased rapidly and those of other fractions increased indicating that supernatant is the site of the last step in the biosynthesis of coenzyme Q.

The foregoing studies point out that the accumulation of coenzyme Q in livers of vitamin A deficient rats results from lowered degradation. It was shown earlier by Hemming et al. (14) that under these conditions the increased coenzyme Q was found in the"ground plasm and free fat fraction of supernatant". Supernatant being the site of synthesis, it appears that the transfer of coenzyme Q from supernatant to other parts of the cell might have been affected under the stress condition. Biosynthesis of coenzyme Q is another case where a mitochondrial lipid component is found to be synthesized outside mitochondria. Wilgram and Kennedy, for example, have shown that the last step in the biosynthesis of phosphatidylcholine, a mitochondrial constituent, is localized in microsomes (15).

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REFERENCES

1. Heaton, F.W., Lowe, J.S. & Morton, R.A., Biochem. J., 67, 208 (1957).
2. Joshi, V.C., Jayaraman, J. & Ramasarma, T., Biochem. J., 88, 25 (1963).

3. Joshi, V.C., Jayaraman, J. & Ramasarma, T., Indian J. exptl. Biol., 1, 113 (1963).
4. Ramasarma, T. & Joshi, V.C., Proceedings of Symposium on Natural Quinones, National Institute of Sciences of India, New Delhi, (1964) (in the press).
5. Morton, R.A. & Phillips, W.E.J., Biochem. J., 73, 416 (1959).
6. Gloor, U. & Wiss, O., Arch. Biochem. Biophys., 83, 216 (1959).
7. Aiyar, A.S., Sulebele, G.A., Rege, D.V. & Sreenivasan, A., Nature, 184, 1867 (1959).
8. Edwin, E.E., Green, J., Diplock, A.T., Bunyan, J., Nature, 186, 725 (1960).
9. Beyer, R.E., Noble, W.M. & Hirschfield, T.J., Can. J. Biochem. & Physiol., 40, 511 (1962).
10. Green, J., Diplock, A.T., Bunyan, J. & McHale, D., Biochim. Biophys. Acta, 78, 739 (1963).
11. Joshi, V.C., Jayaraman, J. & Ramasarma, T., Biochem. J., 92, 9c (1964).
12. Sheshadri Sastri, P., Jayaraman, J. & Ramasarma, T., Nature, 189, 577 (1961).
13. Stoffel, W. & Martius, C., Biochem. Z., 333, 440 (1960).
14. Hemming, P.W., Pennock, J.F. & Morton, R.A., Biochem. J., 68, 29p (1958).
15. Wilgram, G.F. & Kennedy, E.P., J. Biol. Chem., 238, 2615 (1963).