Some Aspects of the Metabolism of Coenzyme Q in the Rat

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Coenzyme Q (ubiquinone) is present in many rat tissues. However, its origin in these tissues, other than liver, is not well established. No deficiency state of coenzyme Q has so far been found. Even when the diet is free of coenzyme Q, tissue concentrations are not affected (Olson, Dialameh & Bentley, 1961; Lawson, Threlfall, Glover & Morton, 1961). The oral administration of coenzyme Q₁₀ to rats increased the concentrations of this compound in liver and to a lesser extent in intestines, but no other tissues tested (heart, kidney, lungs, muscle) showed any change (Lawson *et al.* 1961). Under the conditions employed by Lawson *et al.* (1961), the absorption of small quantities by the tissues will not be detected and also, if a steady-state concentration of coenzyme Q is maintained, no changes would be observed. An attempt has therefore been made to study the distribution of coenzyme Q in rat tissues, including the foetus, by administering [¹⁴C]coenzyme Q₁₀ orally, intracardially and intraperitoneally. Further, the capacity of the rat tissues for the synthesis of coenzyme Q from [2-¹⁴C]mevalonic acid was also investigated. Our results support the view that coenzyme Q in extrahepatic tissues does not originate from liver or blood plasma and that these tissues are capable of synthesizing coenzyme Q.

EXPERIMENTAL

Materials. [¹⁴C]Coenzyme Q₁₀, labelled in the first two carbon atoms of the side chain (specific radioactivity 10.0 µC/mg.) and [2-¹⁴C]mevalonic acid (specific radioactivity 25.8 µC/mg.) were as described by Jayaraman & Ramasarma (1963b).

The rat diet consisted of casein (20%), groundnut oil (5%), cane sugar (10%), corn starch (60%), together with salt mixture (5%) and vitamins as given by the United States Pharmacopeia XIII.

Methods. Pregnancy was timed by the method of Campbell & Kosterlitz (1949). [¹⁴C]Coenzyme Q was administered orally, intracardially or intraperitoneally. Orally it was given dissolved in oil, and for injections an emulsion in ethanol-water (1:5, v/v) was used. [2-¹⁴C]-Mevalonic acid was given orally or intraperitoneally in aqueous solution.

Saponification of tissues was carried out for 20 min. in ethanol (25 ml./g. of tissue) under reflux with aq. 40% (w/v) sodium hydroxide (1.0 ml./g. of tissue) in the presence of ethanolic 20% (w/v) pyrogallol (1.0 ml./g. of tissue).

The unsaponifiable lipids were extracted with light petroleum (b.p. 40–60°). Details of the column chromatography on deactivated alumina are given by Jayaraman & Ramasarma (1961). Coenzyme Q was determined from the change in extinction at 275 mµ on the addition of borohydride to a solution in ethanol. Sterols were determined by the method of Stadtman (1957).

The radioactivity measurements were made as described by Jayaraman & Ramasarma (1963b). The background counting rate was about 25 counts/min. Rates of less than 50 counts/min. were considered insignificant and such samples are marked with asterisks in the Tables. The values reported represent averages of at least two independent determinations, which agreed within 5–10%.

RESULTS

Distribution of [¹⁴C]coenzyme Q in rat tissues after different methods of administration. The pattern of distribution of radioactivity in the unsaponifiable lipids after the administration of [¹⁴C]coenzyme Q by different methods is given in Table 1. Liver was the only tissue that had a significant amount of radioactivity. Even after 24 hr., liver retained about 6% of both oral and intracardial doses.

Table 1. *Distribution in tissues and excretion of administered [¹⁴C]coenzyme Q₁₀ in the rat*

Each rat was given 1 µC of [¹⁴C]coenzyme Q₁₀ and killed after 24 hr. Radioactivity was determined in the unsaponifiable lipids of the tissues, in the light petroleum-extractable lipids of dried faeces and urine, and also in the aqueous suspensions of the lipid-free residues from faeces and urine. (Faeces and urine were collected between 0 and 24 hr.) Experimental details are given in the text. The numbers of rats in each group are given in parentheses. For the meaning of asterisks, see text.

Tissue	Percentage of the dose administered		
	Oral (5)	Intra- cardial (4)	Intra- peritoneal (2)
Liver	6.28	5.50	0.15
Spleen	0.10	0.50	*
Heart, kidney, uterus, brain and intestine	*	*	*
Faeces: lipids	45.60	1.87	0.53
lipid-free residue	3.13	*	*
Urine: lipids	*	*	*
lipid-free residue	0.55	2.37	0.15

When the compound was injected intraperitoneally, however, the deposition in the liver was very low (0.15%). Spleen picked up slight radioactivity when [^{14}C]coenzyme Q_{10} was given orally or intracardially but not when it was administered intraperitoneally. Other tissues tested (heart, kidney, uterus, brain and small intestine) had no measurable radioactivity associated with their unsaponifiable lipids.

When the compounds were given orally, about half of the total radioactivity was excreted in the faeces, mostly in the lipid fraction and probably representing unabsorbed [^{14}C]coenzyme Q. The relatively low radioactivity of urines was in the non-lipid fraction.

Identification of the radioactive compound in rat liver. The unsaponifiable lipids (oral: 11.4 mg., 4044 counts/min.; intracardial: 12.6 mg., 15 300 counts/min.) of livers of rats treated with [^{14}C]coenzyme Q_{10} were fractionated on columns containing 10 g. of 4% deactivated alumina. In

both cases nearly 80% of the radioactivity was eluted in ether-light petroleum (1:20, v/v), the solvent that normally elutes coenzyme Q. Thus the administered compound was present mainly as unchanged coenzyme Q. There was no measurable radioactivity in ether-light petroleum (1:10, v/v), which elutes ubiquinomenol.

Disappearance of [^{14}C]coenzyme Q from blood. The compound was injected intracardially into rats. Samples (0.5–1.0 ml.) were withdrawn from the heart over a period of 21 min. and the radioactivity in the total lipids in the samples was determined. Almost all of the radioactivity was removed from the blood stream within a few minutes of injection (Table 2).

Retention of [^{14}C]coenzyme Q by rat tissues. Rats were given [^{14}C]coenzyme Q intracardially and killed at intervals up to 48 hr. The tissues were removed and saponified, and the radioactivity in the unsaponifiable lipids was determined. Radioactivity was found only in liver and spleen (Table 3) and the former contained about 75% of the dose 30 min. after injection. However, at the end of 2 hr., most of this radioactivity had disappeared and only 4–5% of the original dose was present in the liver. Thereafter, the radioactivity remained steady for about 24 hr., and then decreased to 1–1.5% at 48 hr. Soon after injection, the spleen had 4–5% of the dose and this radioactivity was also lost rapidly. Heart, kidney and small intestine showed slight radioactivity in the very early stages, but none after 1 hr.

Excretion in the faeces collected between 0 and 24 hr. accounted for about 2% of the dose. This was mainly in the lipid fraction. Between 24 and 48 hr., the excretion in the faeces represented 13% of the total dose, and all of the radioactivity was in the non-lipid fraction.

Appearance of coenzyme Q during embryonic development in the rat. The uterine sacs of rats at different stages of pregnancy were removed and

Table 2. *Disappearance of [^{14}C]coenzyme Q from blood*

Each rat was given 1 μC of [^{14}C]coenzyme Q intracardially, and blood was taken from the heart about five times from each rat at the times indicated. The results given are averages of at least two determinations. Total lipids were extracted from 0.5 ml. of blood in each case and the radioactivity was determined. Experimental details are given in the text.

Time (min.)	Radioactivity (counts/min./ml. of blood)
0.5	23 650
1	13 150
4	9 340
5	250
7	218
12	204
15	164
18	30
21	*

Table 3. *Retention of intracardially administered [^{14}C]coenzyme Q by rat tissues*

Each rat was given 1 μC of [^{14}C]coenzyme Q and killed at the times stated. Radioactivity was determined in the unsaponifiable lipids of the tissues, in the light petroleum-extractable lipids of the faeces, and in the dried residue of the urine. (Faeces and urine were collected between 0 and 24 hr. and between 24 and 48 hr.) Experimental details are given in the text.

Time after injection	No. of rats	Percentage of total dose administered					
		Liver	Spleen	Heart	Kidney	Faeces	Urine
7 min.	2	29.93	4.76	0.303	0.239	—	—
30 min	10	74.87	—	*	*	—	—
1 hr.	4	43.35	4.43	*	*	—	—
2 hr.	3	4.06	2.22	*	*	—	—
4 hr.	2	2.80	—	*	*	—	—
6 hr.	2	4.50	—	*	*	—	—
12 hr.	2	2.74	0.18	*	*	—	—
24 hr.	4	5.50	0.50	*	*	1.87	2.37
48 hr.	3	1.38	0.09	*	*	12.80	8.40

the fetuses separated from the uterine tissue. Total lipids were extracted from the fetuses with ethanol-ether (3:1, v/v) and their coenzyme Q contents were determined. The amount of coenzyme Q/fetus increased during development from about 1 μ g. on the fifth day to about 50 μ g. on the twentieth day (Table 4). The following experiment shows that coenzyme Q is supplied through the mother's blood. A summary of this work has appeared (Jayaraman & Ramasarma, 1963a).

Pregnant rats were given [14 C]coenzyme Q₁₀ intracardially and orally, and were killed at the end of 30 min. and 24 hr. respectively. The fetuses

were removed from the uterine sac, the total lipids were extracted and the radioactivity was determined. The radioactivity in the unsaponifiable lipids of liver and the total lipids of fetuses and blood of such animals were also determined (Tables 5 and 6). Small but significant amounts of radioactivity were taken up by the fetuses at various stages of development. This is noteworthy because the fetus is the only other tissue besides liver and spleen that appears to take up administered coenzyme Q.

Incorporation of [2- 14 C]mevalonic acid into the unsaponifiable lipids of rat tissues. The results presented so far suggest that exogenous coenzyme Q is exclusively deposited in the liver and, although metabolized by liver, is not distributed to other tissues. Other rat tissues must therefore possess the capacity to synthesize coenzyme Q. The synthetic activity of certain tissues has been measured in terms of their ability to utilize mevalonic acid, a precursor of coenzyme Q.

[2- 14 C]Mevalonic acid was injected into rats intraperitoneally and the rats were killed after 6 hr. The unsaponifiable lipids were obtained from liver, heart, kidneys, spleen and brain, and fractionated on columns of 4% deactivated alumina to separate coenzyme Q and sterols. In all cases the ether-light petroleum (1:20, v/v) fraction showed a radioactive peak which corresponded to that of coenzyme Q (Table 7).

Attempts were made to establish that [2- 14 C]-mevalonic acid can be incorporated into coenzyme Q of rat fetuses. [2- 14 C]Mevalonic acid was given orally to pregnant rats aged between 6 and 12 days, and after 4 hr. the fetuses were separated and their unsaponifiable lipids obtained. However, when these samples were treated with digitonin, the low amount of radioactivity was removed in the sterol fraction.

DISCUSSION

The rapid disappearance of intracardially administered coenzyme Q₁₀ from the blood of rats and its almost exclusive deposition in liver and spleen follow the general pattern observed for injected fat emulsions, which may be attributed to the reticulo-endothelial system of these tissues. The important point, however, is that whatever the method of administration the radioactive compound was not incorporated in any other tissues tested even after 48 hr. Lawson *et al.* (1961) fed unlabelled coenzyme Q₁₀ to rats and found that the concentration of coenzyme Q increased in liver and intestine, but not in heart, kidney, lungs and muscle, when these were examined 24 hr. after dosing. Threlfall & Glover (1962) confirmed this observation by using uniformly labelled [14 C]-coenzyme Q₁₀. Though our results confirm the

Table 4. *Changes in coenzyme Q content of rat fetuses during development*

Experimental details are given in the text.

Approximate age (days)	No. of samples analysed	Average wt. of fetus (g.)	Coenzyme Q content (μ moles/fetus)
5-6	8†	0.06	1.1
14-15	7	1.80	29.0
18-19	6	3.00	27.8
20-21	7	4.07	56.8

† Eight were pooled for analysis.

Table 5. *Incorporation of intracardially administered [14 C]coenzyme Q into rat fetuses*

Each rat was given 1 μ C of [14 C]coenzyme Q intracardially and killed after 30 min. Radioactivity was determined in the total lipids of fetuses and blood samples and in the unsaponifiable lipids of liver and uterus. Experimental details are given in the text.

Approximate age (days)	Radioactivity (counts/min.)			
	Fetuses	Blood	Liver	Uterus
3-4	—	*	72 800	45†
5-6	31	*	63 300	87
15-16	100	*	82 000	180

† Including small dot-like fetuses.

Table 6. *Incorporation of orally administered [14 C]coenzyme Q into rat fetuses*

Each rat was given 1 μ C of [14 C]coenzyme Q orally and killed after 24 hr. Radioactivity was determined in the total lipids of fetuses and blood, and in the unsaponifiable lipids of liver. Experimental details are given in the text.

Approximate age (days)	Radioactivity (counts/min.)		
	Fetuses	Blood†	Liver
5-6	45	159	3000
7-8	79	33	3420
11-12	300	60	5640

† The total volume of blood in the rat was assumed to be 10 ml.

Table 7. *Incorporation of [2-¹⁴C]mevalonic acid into the unsaponifiable lipids of rat tissues*

Each rat was given 10 μ c of [2-¹⁴C]mevalonic acid intraperitoneally and killed after 6 hr. The unsaponifiable lipids of the tissues from eight rats were chromatographed on 4% deactivated alumina to separate coenzyme Q and sterols, and the radioactivity in these compounds was determined. Experimental details are given in the text. The values given in the Table are calculated per rat.

Tissue	Radioactivity (counts/min.)			Sp. radioactivity	
	Unsaponifiable lipids	Coenzyme Q fraction	Sterol fraction	(counts/min./ μ m-mole of coenzyme Q)	(counts/min./mg. of sterol)
Liver	27 825	970	14 750	1 095	1 370
Heart	640	23	275	577	242
Kidney	22 750	532	16 635	11 650	2 218
Spleen	2 200	10	2 075	5 070	4 580
Brain	289	—	—	—	—

uptake of administered [¹⁴C]coenzyme Q by liver, no radioactivity was found in the unsaponifiable lipids of small intestine. However, not all of the radioactive compound administered to the rat is accounted for in the unsaponifiable lipids (Table 1). The saponifiable matter showed appreciable amounts of radioactivity in the liver and small intestine.

The fate of administered coenzyme Q is in marked contrast with that of vitamin K₁. Taylor, Miller, Jaques & Spinks (1956) found that, when [Me-¹⁴C]vitamin K₁ was administered to rats orally, intramuscularly or intrajugularly, the compound was distributed in all tissues, including blood. About 75% and 15% of the radioactivity in liver and small intestine respectively could be accounted for as unchanged vitamin K₁.

In our experiments, liver took up about 75% of the dose within 30 min. of intracardial injection, but only about 5% and 1% were left after 2 hr. and 48 hr. respectively. During this period, neither blood nor the tissues (heart, kidney, uterus, brain and intestine) appear to have mobilized the hepatic coenzyme Q, since no radioactivity was found in them. Although the foetus is shown to take up blood coenzyme Q, it is apparent that hepatic coenzyme Q is not its source. Rudney & Sugimura (1961) could not detect any radioactivity in the serum after injecting [¹⁴C]coenzyme Q₆ intravenously into rats. Threlfall & Glover (1962) doubted the possibility that heart and kidneys of the rat take up coenzyme Q from plasma. These observations suggest a lack of internal transport of coenzyme Q₁₀ in the rat from liver to other tissues.

It is known that lipid components, e.g. vitamins A, E and K and cholesterol, pass through the placenta (Hagerman & Vilee, 1960). It would appear that blood coenzyme Q can also pass the placental barrier and so be available to the foetus. It is perhaps significant that rat blood contains detectable amounts of radioactivity 24 hr. after

the oral administration of [¹⁴C]coenzyme Q. The slow absorption of dietary coenzyme Q will presumably maintain a low concentration of coenzyme Q in the blood. Therefore, dietary coenzyme Q assumes an indirect role in embryonic development. Further discussion of this point has been presented by Jayaraman & Ramasarma (1963a).

Lawson *et al.* (1961) observed that, when [¹⁴C]-acetate was administered orally or intraperitoneally to rats, the coenzyme Q in liver, skin and intestine was labelled. In these tissues, the specific radioactivities of coenzyme Q and sterols ran parallel to each other. Lawson *et al.* (1961) concluded that skin and intestine of rat can also synthesize coenzyme Q. In the present experiments, [2-¹⁴C]mevalonic acid administered orally to rats was incorporated into coenzyme Q of heart, kidney and spleen in addition to that of liver. The two observations that the transport of preformed coenzyme Q from liver to other tissues is absent and that coenzyme Q of kidney and spleen had higher specific radioactivity than that of liver suggest that the radioactive coenzyme Q of these tissues must have been endogenously synthesized.

The presence of radioactivity in the saponifiable matter of tissues (e.g. small intestine), and also in the non-lipid portions of the urine and faeces, indicates a breakdown of [¹⁴C]coenzyme Q₁₀ in the animal body. When administered orally, only about 45% of the dose was excreted through the faeces and of this over 90% was in the lipid fraction. Of the 55% of the dose that was absorbed only part was recovered in the unsaponifiable lipids of liver. The rest of the radioactivity, presumably, is present in the saponifiable matter. Quantitative results on this aspect could not, however, be obtained because of interference from the resinous material formed during saponification in the presence of pyrogallol.

One deficiency in the experimental approach calls for discussion. The homologue that we have used is coenzyme Q₁₀. In liver only 10–15% of the

coenzyme Q is coenzyme Q₁₀, the greater part being coenzyme Q₉. Whether coenzyme Q₉ would behave differently in the rat body will remain uncertain until [¹⁴C]coenzyme Q₉ becomes available. However, two arguments can be advanced in favour of the validity of our experiments. The homologues coenzyme Q₁₀ and coenzyme Q₉ are both synthesized in rat liver (Wiss, Gloor & Weber, 1961), and, in addition, rat brain contains a relatively high concentration of coenzyme Q₁₀ (Green, 1961). If transport from liver were to be the source of coenzyme Q in other tissues, then coenzyme Q₁₀ should also have been incorporated into brain.

SUMMARY

1. Administration of [¹⁴C]coenzyme Q₁₀ by oral or intracardial routes to rats resulted in its exclusive incorporation into liver. Although this radioactivity rapidly decreased, it was not transferred to other tissues even after 48 hr.

2. The greater part of the radioactivity in the unsaponifiable lipids of liver was in the form of unchanged coenzyme Q₁₀, even after 24 hr. Under these conditions, administered [¹⁴C]coenzyme Q₁₀ was not converted into its cyclic isomer, ubiquinomenol.

3. The coenzyme Q content of rat fetuses increased during development. Blood coenzyme Q can pass through placenta and become incorporated into the fetuses.

4. Injected [¹⁴C]coenzyme Q₁₀ is rapidly removed from rat blood. Dietary coenzyme Q seems to be the source of blood coenzyme Q and therefore assumes indirect importance in embryonic development.

5. Several rat tissues are capable of synthesizing [¹⁴C]coenzyme Q from [2-¹⁴C]mevalonic acid.

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Physicochemical Properties of Ox-Brain Gangliosides

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Gangliosides are the group of sialomucolipids that are derived from cerebral tissues. Methods of preparation and purification of gangliosides and recent advances in the elucidation of their chemical structure have been reviewed by McIlwain (1961a) and Rapport & Norton (1962). Fractionation of gangli-

osides has demonstrated the existence of several molecular species differing in their chemical composition. The differences between species, however, are relatively small and simple molar proportions of the chemical constituents have been assigned to each. Thus each has been shown to have a polar