Intracellular Distribution and Biosynthesis of Ubiquinone in Rat Liver in Carbon Tetrachloride Liver Injury

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The fundamental metabolic deviation by which carbon tetrachloride administration results in fatty degeneration of liver is not yet clear. In recent reports on the changes in composition and function of the fatty liver cell particular attention has been paid to the mitochondria. A damage to mitochondrial integrity is accompanied by major biochemical alterations (Dianzani, 1954, 1955, 1956; Dianzani & Viti, 1955; Kasbekar, Lavate, Rege & Sreenivasan, 1959). Inhibition of certain enzymes (Dianzani, 1953) and activation of others (Lehninger, 1951; Berthet & de Duve, 1951; Schneider & Hogeboom, 1952*a*, *b*; Hogeboom & Schneider, 1953) latent in fresh mitochondria, especially of adenosine triphosphatase (Kielley & Kielley, 1951; Potter & Recknagel, 1951; Copenhaver & Lardy, 1952; Lardy & Wellman, 1952, 1953) have been reported.

Dianzani & Viti (1955) have observed decreases in both cytochrome *c* and pyridine nucleotides in fatty livers. Work in this Laboratory (Nadkarni, Wagle & Sreenivasan, 1957) has further shown that the biosynthesis *in vivo* of pyridine nucleotides from intraperitoneally administered nicotinamide is less in the carbon tetrachloride-treated animals. A decrease in hepatic coenzyme A has also been reported in fatty degeneration (Severi & Fonnesu, 1956; Heim, Leuschner & Ott, 1956). In view of the changes in cytochrome *c* and pyridine nucleotides, and in coenzyme A, which is known to be

necessary for the biosynthesis of ubiquinone (cf. Aiyar, Sulebele, Rege & Sreenivasan, 1959; Aiyar & Sreenivasan, 1961), a study of the alterations in ubiquinone levels in fatty livers is of interest.

In this paper we present data on the intracellular distribution of ubiquinone and on its biosynthesis *in vitro* by liver slices from labelled precursors.

EXPERIMENTAL

Adult male albino rats (Wistar strain) reared on the laboratory stock diet (cf. Kasbekar *et al.* 1959) and weighing 140–150 g. were administered intraperitoneally with 2·0 ml. of carbon tetrachloride/kg. body wt. The control animals were given corresponding quantities of distilled water. Where protection by vitamin B_{12} was studied, the vitamin (10 μg./animal) was given intraperitoneally 3 hr. before carbon tetrachloride injection, since maximal protection by the vitamin against fat accumulation has been shown under these conditions (Rege, 1953). The animals were killed at intervals of 0, 8, 24 and 48 hr. after administration of carbon tetrachloride. The livers were excised, chilled and homogenized in a previously chilled Potter–Elvehjem-type glass homogenizer to 10% suspensions in cold iso-osmotic (0·25 M) sucrose. Fractionation of the suspensions into cellular components was achieved by differential centrifuging. Nuclei and mitochondria were sedimented in an International PR-2 refrigerated centrifuge as described by Schneider & Hogeboom (1950), and microsomes and supernatant were separated in a Spinco model L preparative ultracentrifuge by the method of Palade & Siekevitz (1955).

Portions of the liver were cut into thin slices with a Stadie tissue slicer (A. H. Thomas Co., U.S.A.) and were incubated in Krebs–Ringer phosphate buffer, pH 7·2 (cf. Kasbekar & Sreenivasan, 1959), containing either [$^{14}C_2$]acetate (10 μC) or [2-^{14}C]mevalonate (10 μC) together with 3 μM-adenosine triphosphate and 60 μM-glucose in a final volume of 4·0 ml. for 3 hr. in Warburg flasks at 37°. At the end of the incubation period, the suspending medium was decanted off, and the slices were washed twice with the buffer and taken for estimation of ubiquinone.

Ubiquinone was estimated in the whole liver, and in cellular fractions, by the spectrophotometric method of Crane, Lester, Widmer & Hatefi (1959) after saponification and purification of the unsaponifiable lipids by chromatography on Brockmann grade III alumina (Festenstein, Lowe & Morton, 1955).

Ubiquinone in the liver slices was isolated by chromatography (Festenstein *et al.* 1955), and the counts were read in a Tracerlab SC-16 windowless gas-flow counter in conjunction with a Tracerlab SC-51 autoscaler. The ubiquinone was later determined spectrophotometrically (Crane *et al.* 1959).

Nitrogen was estimated by a micro-Kjeldahl method (Umbreit, Burris & Stauffer, 1946).

RESULTS AND DISCUSSION

Changes in ubiquinone (expressed/100 mg. of liver N) due to carbon tetrachloride administration with and without vitamin B_{12} protection are presented in Table 1. Carbon tetrachloride administration results only in an insignificant decrease in ubiquinone concentration of liver, whereas vitamin B_{12} exerts a protection against this change. Even though the total ubiquinone in liver per unit of nitrogen is only slightly decreased in the fatty livers, there is a marked reduction in the mitochondrial fraction, with concomitant increase in the supernatant fraction (Table 2). Similar alterations in the distribution of other mitochondrial components are known (Dianzani, 1955; Dianzani & Viti, 1955; Kasbekar & Sreenivasan, 1956). Vitamin B_{12} affords almost complete protection against this alteration in the distribution of ubiquinone. This observation would further substantiate the view that vitamin B_{12} exerts its protection against carbon tetrachloride injury through a reversal of the changes in the mitochondrion (Kasbekar *et al.* 1959).

Studies on the incorporation of [$^{14}C_2$]acetate and [2-^{14}C]mevalonate into ubiquinone by liver slices (Table 3) reveal a greatly depressed incorporation by the injured slices. An explanation for this may be found in the reported decreases in coenzyme A (Severi & Fonnesu, 1956; Heim *et al.* 1956) and adenosine triphosphate (Dianzani, 1955) in fatty livers. Although coenzyme A is known to be involved in the biosynthesis of the isoprenoid side chain of ubiquinone from acetate (Aiyar *et al.* 1959; Aiyar & Sreenivasan, 1961), evidence has become available in recent years that maintenance of

Table 1. *Effect of carbon tetrachloride administration on hepatic stores of ubiquinone*

Adult male rats weighing 140–150 g. and reared on the laboratory stock diet were administered intraperitoneally with 2·0 ml. of carbon tetrachloride/kg. body wt. To the group administered with vitamin B_{12}, the vitamin was given 3 hr. before carbon tetrachloride. Results are averages of four independent determinations ± S.E.M.

Time after administration (hr.)	Nitrogen (mg./g.)		Ubiquinone (μg./100 mg. of N)	
	Without vitamin B_{12}	With vitamin B_{12}	Without vitamin B_{12}	With vitamin B_{12}
0	32·8 ± 1·7	32·8 ± 2·1	367 ± 21	352 ± 16
8	28·6 ± 1·8	30·6 ± 0·6	329 ± 16	339 ± 11
24	24·7 ± 3·1	31·9 ± 1·2	318 ± 19	346 ± 25
48	29·4 ± 1·6	31·4 ± 1·8	308 ± 24	341 ± 22

Table 2. *Intracellular distribution of ubiquinone in carbon tetrachloride liver injury*

Results are averages of four independent determinations ± S.E.M.

Group	Ubiquinone (μg./100 mg. of N of whole liver)					
	Whole liver	Nuclei	Mitochondria	Microsomes	Supernatant	Percentage of total in mitochondria
Normal	367±21	82±7	148±11	59±4	25±3	40·0
Carbon tetrachloride administered	306±24	79±3	84±7	41±7	72±6	27·5
Carbon tetrachloride administered with vitamin B_{12} protection	341±22	74±9	126±12	66±11	37±6	37·0

Table 3. *Biosynthesis of ubiquinone from labelled precursors by carbon tetrachloride-injured liver slices*

Liver slices (500 mg.) were incubated in a system containing acetate (10 μc) or mevalonate (10 μc) with 3 μM-ATP, and 60 μM-glucose in 4·0 ml. of Krebs–Ringer phosphate buffer, pH 7·2, for 3 hr. Results are averages of four independent determinations ± S.E.M.

Group	Ubiquinone synthesized			
	From [$^{14}C_2$]acetate		From [2-^{14}C]mevalonate	
	(Counts/min.)	Specific activity (counts/min./mg.)	(Counts/min.)	Specific activity (counts/min./mg.)
Normal	204±21	746±94	341±61	1128±101
Carbon tetrachloride administered	118±32	316±56	239±17	696±47

adequate concentrations of adenosine triphosphate is essential for utilization of mevalonic acid for synthesis of isoprenoids through a phosphorylated intermediate (Wright & Englert, 1960; Wright, Li & Trager, 1960; Gosselin & Podber-Wagner, 1961).

SUMMARY

1. Carbon tetrachloride administration results in a decrease in the mitochondrial ubiquinone and an increase in the ubiquinone of the supernatant fraction.

2. Administration of vitamin B_{12} 3 hr. before carbon tetrachloride affords significant protection against this alteration in intracellular distribution of ubiquinone.

3. The incorporation of [$^{14}C_2$]acetate and [2-^{14}C]-mevalonate into ubiquinone by liver slices is decreased by carbon tetrachloride administration.

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