soluble RNA-amino acid complex and microsomes and only to a lesser degree in the steps involving amino acids activation and transport. This step requires GTP as a cofactor. It is thus conceivable that an increased synthesis of proteins may be accompanied by an increased turnover of guanine nucleotides. Increase in 5'-nucleotidase and guanase activity of the cells as brought about by growth-hormone administration may be connected with an increased turnover of guanine nucleotides.

Growth-hormone administration is also seen to cause a decrease in xanthine-oxidase activity. Bergel, Bray, Haddow & Lewin (1957) have ascribed a key role to this enzyme in the purine cycle. Its activity is seen to decrease in phases of accelerated growth (Lamirande *et al.* 1958).

SUMMARY

1. Activities of 5'-nucleotidase, adenase, guanase and xanthine oxidase have been estimated in livers from normal and growth-hormone-treated rats.

2. Increased 5'-nucleotidase and guanase activities were found after growth-hormone administration; xanthine-oxidase activity fell.

Part of the results reported here are included in the M.Sc. thesis of N. C. Panda. Gift of Antuitrin-G from Parke, Davis and Co. Ltd. is gratefully acknowledged.

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Biochem. J. (1962) **82**, 179

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 B₁₂ 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 (0.25M) 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 [¹⁴C₂]acetate (10 µC) or [2-¹⁴C]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 (Festenstien, Lowe & Morton, 1955).

Ubiquinone in the liver slices was isolated by chromatography (Festenstien *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 B₁₂ protection are presented in Table 1. Carbon tetrachloride administration results only in an insignificant decrease in ubiquinone concentration of liver, whereas vitamin B₁₂ 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 B₁₂ affords almost complete protection against this alteration in the distribution of ubiquinone. This observation would further substantiate the view that vitamin B₁₂ 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 [¹⁴C₂]acetate and [2-¹⁴C]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 B₁₂, 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 $\pm$ S.E.M.

Group	Ubiquinone ($\mu\text{g./100 mg. of N of whole liver}$)					Percentage of total in mitochondria
	Whole liver	Nuclei	Mitochondria	Microsomes	Supernatant	
Normal	367 $\pm$ 21	82 $\pm$ 7	148 $\pm$ 11	59 $\pm$ 4	25 $\pm$ 3	40.0
Carbon tetrachloride administered	306 $\pm$ 24	79 $\pm$ 3	84 $\pm$ 7	41 $\pm$ 7	72 $\pm$ 6	27.5
Carbon tetrachloride administered with vitamin B ₁₂ protection	341 $\pm$ 22	74 $\pm$ 9	126 $\pm$ 12	66 $\pm$ 11	37 $\pm$ 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 $\pm$ 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 $\pm$ 21	746 $\pm$ 94	341 $\pm$ 61	1128 $\pm$ 101
Carbon tetrachloride administered	118 $\pm$ 32	316 $\pm$ 56	239 $\pm$ 17	696 $\pm$ 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 B₁₂ 3 hr. before carbon tetrachloride affords significant protection against this alteration in intracellular distribution of ubiquinone.

3. The incorporation of [¹⁴C₂]acetate and [2-¹⁴C]-mevalonate into ubiquinone by liver slices is decreased by carbon tetrachloride administration.

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Content and Intracellular Distribution of Ubiquinone in the Rat in Experimental Thyrotoxicosis

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A functional relationship between thyroid activity and respiratory metabolism in animals has been suggested by many authors. Thus there have been reports of increased rates of oxygen consumption (Rossiter, 1940; Dunne & Tapley, 1960) and, more specifically, of succinate oxidation (Gemmill, 1951; Wolff & Ball, 1957) in tissues treated with the hormone. The first report of a direct influence of the thyroid hormone on concentrations of intracellular respiratory carriers came from Tipton, Leath, Tipton & Nixon (1945-46), who observed an increase in cytochrome oxidase in the hyperthyroid animal. Drabkin (1950) has observed an enhancement of cytochrome *c* stores in tissues in hyperthyroidism and has suggested that the increase in respiratory metabolism is a direct effect of the increase in cytochrome *c*, which probably is the rate-limiting component in the electron-transfer chain. Similar increases of cytochrome *c* and cytochrome oxidase in hyperthyroidism have since been found by others (Nikkila & Pitkanen, 1959; C. Bhuvaneshwaran & A. Sreenivasan, unpublished work). Thyroxine also induces an increase in hepatic concentration of coenzyme A (Tabachnik & Bonnycastle, 1954; Turchetto, Sanguinetti & Rossi, 1955) and an enhancement of different coenzyme A-dependent reactions (cf. Barker, 1951; Solomon & Dowling, 1960; Kritchevsky, 1960). We have observed that in pantothenic acid-deficient rats there is a decrease in liver ubiquinone (Aiyar, Sulebele, Rege & Sreenivasan, 1959; Aiyar & Sreenivasan, 1961) and also a lowered incorporation of [^{14}C]acetate and [2- ^{14}C]mevalonate into liver ubiquinone (A. S. Aiyar & A. Sreenivasan,

unpublished work). These experiments appear to indicate the involvement of coenzyme A in the biosynthesis of ubiquinone.

In view of these reports a study of the effect of experimental thyrotoxicosis on liver concentrations of ubiquinone, a recently recognized member of the electron-transport chain (Crane, Hatefi, Lester & Widmer, 1957), appeared to be of interest. Observations on the intracellular distribution of ubiquinone in the hyperthyroid rat liver, and the effect of vitamin B_{12} supplementation thereon are presented and discussed.

EXPERIMENTAL

Male rats, Wistar strain, were rendered thyrotoxic by feeding a purified 10% casein ration devoid of vitamin B_{12} and containing 0.10% of iodinated casein (Protomone, Cerophyll Laboratories, Kansas, Mo., U.S.A.) (cf. Kasbekar, Lavate, Rege & Sreenivasan, 1959). Control rats without iodinated casein as well as two more groups with vitamin B_{12} supplementation (200 $\mu\text{g./kg.}$ of diet) of the basal and the iodinated-casein-supplemented diets were also maintained. At the end of 8 weeks, when the animals fed the iodinated casein showed obvious symptoms of thyrotoxicosis, the animals were exsanguinated. The livers were homogenized and subcellular fractions were prepared as described in the preceding paper (Aiyar & Sreenivasan, 1962).

Vitamin B_{12} in liver homogenates was determined microbiologically by using *Euglena gracilis* as test organism (Hoff-Jorgensen, 1954), and succinoxidase was determined manometrically by the method of Schneider & Potter (1943). Ubiquinone in whole liver and in subcellular fractions was estimated as in the preceding paper (Aiyar & Sreenivasan, 1962).