

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in hyperthyroidism have since been found by others (Nikkila & Pitkanen, 1959; C. Bhuvaneshwaran & A. Sreenivasan, unpublished work).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]₂acetate 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unpublished work).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 B₁₂ supplementation thereon are presented and discussed.

EXPERIMENTAL

Male rats, Wistar strain, were rendered thyrotoxic by feeding a purified 10% casein ration devoid of vitamin B₁₂ 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 B₁₂ supplementation (200 $\mu\text{g.}/\text{kg.}$ 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 B₁₂ 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).

Table 1. *Changes in liver stores of ubiquinone in thyrotoxicosis*

Results are averages of six independent determinations \pm s.e.m. and are expressed on a fresh-weight basis.

Group	Vitamin B ₁₂ (μ mg./g.)	Ubiquinone (μ g./g.)	Succinoxidase activity (μ l. of O ₂ /hr./g.)
10% Casein diet	53 \pm 6.0	123 \pm 11	962 \pm 49
10% Casein diet + vitamin B ₁₂	87 \pm 11.0	128 \pm 8	1024 \pm 33
10% Casein diet + iodinated casein	31 \pm 4.0	221 \pm 21	1270 \pm 64
10% Casein diet + iodinated casein + vitamin B ₁₂	78 \pm 7.0	198 \pm 13	1164 \pm 22

Table 2. *Intracellular distribution of ubiquinone in rat liver in thyrotoxicosis*

The supernatant fraction was freeze-dried before saponification in the assay procedure for ubiquinone. Results are averages of six independent determinations \pm s.e.m. and are expressed on a fresh-weight basis.

Group	Ubiquinone (μ g./g.)					Percentage of total in mito- chondria
	Whole liver	Nuclei	Mito- chondria	Micro- somes	Super- natant	
10% Casein diet	123 \pm 11	31 \pm 4	49 \pm 6	18 \pm 2	8 \pm 1	40
10% Casein diet + iodinated casein	221 \pm 21	54 \pm 9	97 \pm 11	29 \pm 7	13 \pm 2	44

RESULTS AND DISCUSSION

In Table 1 are presented data on vitamin B₁₂, ubiquinone and succinoxidase in liver. The hyperthyroid animals show a twofold increase in the liver ubiquinone concentration, accompanied by a significant increase in the succinoxidase activity. The increase in ubiquinone is similar to the reported increases in cytochrome *c* and cytochrome oxidase (Tipton *et al.* 1945-46; Drabkin, 1950; Nikkila & Pitkanen, 1959), and it appears probable that the increased metabolic rate necessitates an increase in all these respiratory carriers. Other work in this Laboratory has shown that, in the hyperthyroid animal, the syntheses of cytochrome *c* and of cytochrome oxidase are favoured at the expense of catalase, which shows a decline (C. Bhuvaneshwaran & A. Sreenivasan, unpublished work).

Sure & Easterling (1950) have shown that vitamin B₁₂ protects rats against thyrotoxicosis, and Kasbekar *et al.* (1959) reported that this protection arises primarily from maintenance of the sulphhydryl reserves of the cell and hence of mitochondrial integrity and function. Vitamin B₁₂ supplementation has also been observed to restore the levels of cytochrome *c* and of cytochrome oxidase to normal in the hyperthyroid animal (C. Bhuvaneshwaran & A. Sreenivasan, unpublished work). The present studies reveal that vitamin B₁₂ has no marked effect on either ubiquinone concentration or succinoxidase activity, even though it causes the liver stores of the vitamin to be replenished (Table 1).

Analysis of subcellular fractions of normal rat liver for ubiquinone (cf. Table 2) shows that 40% is present in the mitochondria whereas 31, 18 and 8% are in the nuclei, microsomes and supernatant fluid respectively. The increase in ubiquinone in thyrotoxicosis is most marked in the mitochondrial fraction, which is also the seat of oxidative metabolism. Green, Søndergaard & Dam (1956) studied the distribution in ox liver of vitamin K, another lipid quinone implicated in electron transport. They found 25% in the mitochondria and 45% in the nuclei.

SUMMARY

1. Thyrotoxicosis results in a twofold increase in ubiquinone accompanied by an elevation of succinoxidase activity in rat liver; the rise in ubiquinone is particularly marked in the mitochondria.

2. Vitamin B₁₂ supplementation has no marked effect on either ubiquinone concentration or succinoxidase activity, in both the control and the thyrotoxic animals.

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REFERENCES

- Aiyar, A. S. & Sreenivasan, A. (1961). *Nature, Lond.*, **190**, 344.
 Aiyar, A. S. & Sreenivasan, A. (1962). *Biochem. J.* **82**, 179.
 Aiyar, A. S., Sulebele, G. A., Rege, D. V. & Sreenivasan, A. (1959). *Nature, Lond.*, **184**, 1867.

- Barker, S. B. (1951). *Physiol. Rev.* **31**, 205.
- Crane, F. L., Hatefi, Y., Lester, R. L. & Widmer, C. (1957). *Biochim. biophys. Acta*, **25**, 220.
- Drabkin, D. L. (1950). *J. biol. Chem.* **182**, 335.
- Dunne, P. B. & Tapley, D. F. (1960). *Nature, Lond.*, **185**, 623.
- Gemmill, C. L. (1951). *Amer. J. Physiol.* **170**, 502.
- Green, J. P., Søndergaard, E. & Dam, H. (1956). *Biochim. biophys. Acta*, **19**, 182.
- Hoff-Jorgensen, E. (1954). *Meth. biochem. Anal.* **1**, 81.
- Kasbekar, D. K., Lavate, W. V., Rege, D. V. & Sreenivasan, A. (1959). *Biochem. J.* **72**, 374.
- Kritchevsky, D. (1960). *Metabolism*, **9**, 984.
- Nikkila, E. A. & Pitkanen, E. (1959). *Biol. Abstr.* **35**, 11.
- Rossiter, R. J. (1940). *J. Endocrin.* **2**, 165.
- Schneider, W. C. & Potter, V. R. (1943). *J. biol. Chem.* **149**, 217.
- Solomon, D. H. & Dowling, J. T. (1960). *Annu. Rev. Physiol.* **22**, 615.
- Sure, B. & Easterling, L. (1950). *J. Nutr.* **42**, 221.
- Tabachnik, I. I. A. & Bonnycastle, D. D. (1954). *J. biol. Chem.* **207**, 757.
- Tipton, S. R., Leath, M. J., Tipton, I. H. & Nixon, W. L. (1945-46). *Amer. J. Physiol.* **145**, 693.
- Turchetto, E., Sanguinetti, F. & Rossi, C. A. (1955). *Boll. Soc. ital. Biol. sper.* **31**, 1324.
- Wolff, E. C. & Ball, E. G. (1957). *J. biol. Chem.* **224**, 1083.
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