Metabolism of Ubiquinone in Relation to Thyroxine Status

By A. R. INAMDAR AND T. RAMASARMA

Department of Biochemistry, Indian Institute of Science,
Bangalore 12, India

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1. Under conditions of thyrotoxicosis induced by feeding rats with iodinated casein, ubiquinone concentration was found to increase in the liver by increased synthesis and by partly decreased catabolism leading to its accumulation. The increased ubiquinone was found primarily in the mitochondrial and supernatant fractions. 2. Supplementing the diet with thyroxine, at less than toxic doses, also increased the synthesis and the concentration of ubiquinone in the liver. 3. In the condition of hypothyroidism obtained by feeding rats with thiouracil the concentration and the synthesis of ubiquinone in the liver showed a small decrease. 4. Synthesis of ubiquinone in liver slices was partially inhibited by addition of thyroxine *in vitro*. Therefore the activation effect on ubiquinone synthesis of excess of thyroxine in the intact animals appears to be by an indirect mechanism.

The concentration of liver ubiquinone in the rat was shown to increase in hyperthyroid conditions obtained by feeding with iodinated casein (Aiyar, Sulebele, Rege & Sreenivasan, 1959), by administering thyroxine (Edwin, Green, Diplock & Bunyan, 1960; Beyer, Noble & Hirschfeld, 1962*a*) and by prolonged exposure to cold environment (Beyer, Noble & Hirschfeld, 1962*b*). Pedersen, Tata & Ernster (1963), however, found that concentrations of ubiquinone were unchanged in short-term experiments where basal metabolic rate was increased by administering small doses of tri-iodothyronine to thyroidectomized rats and therefore concluded that thyroxine-induced increase in ubiquinone was probably a result and not the cause of increased basal metabolic rate. Also, Brubacher & Wiss (1961) reported that feeding thiouracil to rats, which is known to produce a hypothyroid condition by inhibiting the endogenous synthesis of thyroxine (Franklin, Chaikoff & Lerner, 1944; Iino, Yamada & Greer, 1961), did not induce any change in liver ubiquinone. But in all these experiments only the ubiquinone concentrations were measured and therefore they do not determine if there was a large turnover of the molecule. We have re-examined the influence of thyroxine status in the rat on the synthesis and catabolism of liver ubiquinone, as tested by the incorporation of radioactive mevalonate. Our results confirm the previous findings of changes in concentration and in addition show that excess of thyroxine increases the synthesis of ubiquinone in the intact animal but is inhibitory when tested with liver slices.

EXPERIMENTAL

Labelled compounds. DL-[2-¹⁴C]Mevalonic acid lactone (2.37 mc/m-mole) was obtained from The Radiochemical Centre, Amersham, Bucks., and was hydrolysed to the acid before use. [¹⁴C]Ubiquinone-10 (labelled in the first two carbon atoms attached to the ring) was a gift from Dr O. Wiss, Hoffmann-La Roche and Co. Ltd., Basle, Switzerland.

Animals, diets and supplements. Male weanling albino rats of the Institute colony, weighing about 50 g., were used. The normal diet consisted of vitamin-free casein (20%), peanut oil (5%), sugar (10%), salt mixture (*U.S. Pharmacopeia*, 1947) (5%) and starch to 100%. Each rat received daily 0.5 ml. of vitamin B complex solution (*U.S. Pharmacopeia*, 1947). Vitamin A (250 µg.), vitamin D (1.5 µg.), α-tocopherol (2 mg.) and vitamin K₃ (50 µg.) were given to each rat weekly. Water and food were supplied *ad lib*.

The animals were rendered thyrotoxic by addition of iodinated casein to the diet (0.3 or 0.5% as specified). Protein content of the diet was reduced to produce thyrotoxic conditions rapidly. Comparison was made with the corresponding control animals fed with a low-protein diet. In the second set of experiments, the animals on the normal diet were given a supplement of extra L-thyroxine either in the diet (30 mg./kg. of diet) or by subcutaneous injection (50 µg. in 0.1 ml. of saline/rat/day). In the third set of experiments, thiouracil (0.3%) was added in the diet. Thyroxine supplements were given to these animals as in the above experiments.

The tracers, mevalonate (2 µC/rat) or ubiquinone (0.5 µC/rat), were given orally and after specified time-intervals the animals were killed by stunning and decapitation, and the tissues removed and processed further.

Six animals were used in each group and mean and standard deviation are given for each analysis. The values are given on the basis of wet weight of the tissue. The liver

weights also are given in appropriate places so that comparison can be made on whole-organ basis. In the experiments on the incorporation of radioactive compounds two animals were used in each group and the average values of independent analysis (within 10% variation) are given. In each experiment, the results of the experimental group are compared with the normal group identically treated except for the supplements and this is necessary in view of the variation of normal values from experiment to experiment.

Tissue-slice experiment. Slices (0.5 mm. thickness) of liver from an adult rat on normal diet were prepared with a tissue slicer (Arthur Thomas and Co., Philadelphia, Pa., U.S.A.). The slices (0.4 g./flask) were suspended in 6 ml. of Krebs-Ringer phosphate buffer, pH 7.4 (Krebs & Henseleit, 1932), containing [2-¹⁴C]mevalonate (2 μ C/flask), and where indicated L-thyroxine (5 μ g./flask), and incubated at 37° for 3 hr. in a metabolic shaker. At the end of the experimental period ethanol (15 ml.) was added, the contents were heated in a boiling-water bath for 3 min. and the whole samples without filtration were processed for the preparation of unsaponifiable lipids.

Unsaponifiable lipids and separation of ubiquinone. The tissues were saponified under reflux in ethanol (20 ml./g. of tissue) with aq. 40% (w/v) sodium hydroxide (1 ml./g. of tissue) in the presence of pyrogallol (0.1 g./g. of tissue) for 20 min. The unsaponifiable lipids were extracted with light petroleum (b.p. 40–60°) and fractionated on a deactivated alumina column, the 'hydrocarbons', ubiquinone and 'sterols' being eluted in light petroleum, 5% (v/v) diethyl ether–light petroleum and 20% (v/v) ether–light petroleum respectively. The 20%-ether fraction was not processed further and was intentionally chosen so as to represent the amount and radioactivity present in 'total sterols'. The recoveries of radioactivity in these fractions were usually about 80% of the total unsaponifiable lipids. Determinations of ubiquinone, 'sterols' and radioactivity in the samples were made as described by Joshi, Jayaraman & Ramasarma (1963a).

In the experiments with radioactive tracers, ubiquinone samples were further purified by thin-layer chromatography on alumina G and then by repeated crystallization to constant specific radioactivity.

Preparation of cellular fractions. The livers (usually about 4–5 g.) were homogenized in a medium (10 ml./g.) of 0.25 M-sucrose (containing 0.01 M-tris, pH 7.4, and 0.005 M-EDTA) in a Potter-Elvehjem homogenizer. The homogenate was separated into nuclear, mitochondrial, microsomal and supernatant fractions by conventional differential centrifugation as described by Schneider & Hogeboom (1950).

RESULTS

Experimental thyrotoxicosis and ubiquinone metabolism. Rats at weanling stage were made thyrotoxic by feeding with iodinated casein (0.5%, w/w) in the diet for different numbers of days depending on the dietary protein concentration. Animals on protein-deficient diet became ill very rapidly and did not survive after 16 days. With 12%- and 20%-protein diets, long time-intervals were required to produce toxic conditions. In all cases, comparisons made with animals on the same protein content in the diet and analysed at each time-interval showed that liver ubiquinone concentration increased progressively with the feeding time (Table 1).

Increased ubiquinone was found only in the liver but not in the kidneys or the intestines (Table 2), a feature similar to findings in vitamin A deficiency (Heaton, Lowe & Morton, 1957) and cold exposure (Aithal, Joshi & Ramasarma, 1968).

Incorporation of radioactive mevalonate into ubiquinone (representing its synthesis) showed a marked increase in the livers of iodinated-casein-fed rats compared with the livers of corresponding normal animals (Table 3). The increase was more marked at the 5 and 10 days experimental stage than at 20 days. Comparison was also made by normalizing the values for ubiquinone for a value of 100 000 counts/min./liver for unsaponifiable lipids and also by specific radioactivity. It can be

Table 1. *Concentration of ubiquinone in livers of rats fed with iodinated casein and different amounts of protein*

Groups of six weanling rats were fed on a diet containing specified amounts of protein and, where mentioned, 0.5% iodinated casein for different numbers of days.

Protein in the diet (%)	Experimental period (days)	Normal		Thyrotoxic (fed with iodinated casein)	
		Liver wt. (g.)	Ubiquinone (μ moles/g.)	Liver wt. (g.)	Ubiquinone (μ moles/g.)
20	10	4.5 $\pm$ 0.3	150 $\pm$ 11	3.7 $\pm$ 0.5	170 $\pm$ 20
	25	5.3 $\pm$ 0.5	140 $\pm$ 14	4.5 $\pm$ 0.6	240 $\pm$ 24
	40	8.3 $\pm$ 0.6	110 $\pm$ 10	5.9 $\pm$ 0.4	280 $\pm$ 25
	60	8.0 $\pm$ 0.8	120 $\pm$ 10	6.0 $\pm$ 0.4	380 $\pm$ 20
	80	8.8 $\pm$ 0.8	160 $\pm$ 13	6.3 $\pm$ 0.7	430 $\pm$ 22
12	10	3.6 $\pm$ 0.4	136 $\pm$ 13	3.3 $\pm$ 0.3	236 $\pm$ 20
	17	4.3 $\pm$ 0.5	125 $\pm$ 14	3.9 $\pm$ 0.2	285 $\pm$ 25
Nil	10	2.9 $\pm$ 0.3	84 $\pm$ 11	1.7 $\pm$ 0.2	210 $\pm$ 20
	16	3.2 $\pm$ 0.2	60 $\pm$ 7	2.9 $\pm$ 0.5	140 $\pm$ 11

Table 2. *Concentration of ubiquinone in liver, intestines and kidney of thyrotoxic rats*

Groups of six weanling rats were fed with a diet containing 12% of casein and, where mentioned, 0.3% of iodinated casein, for 34 days.

Tissue	Normal		Thyrotoxic (fed with iodinated casein)	
	Wt. (g.)	Ubiquinone (μ moles/organ)	Wt. (g.)	Ubiquinone (μ moles/organ)
Liver	5.5 $\pm$ 0.6	915 $\pm$ 64	5.0 $\pm$ 0.5	2190 $\pm$ 320
Intestines	6.3 $\pm$ 0.6	260 $\pm$ 7	6.0 $\pm$ 0.4	250 $\pm$ 6
Kidney	1.40 $\pm$ 0.04	120 $\pm$ 11	1.20 $\pm$ 0.07	125 $\pm$ 14

Table 3. *Effect of feeding rats with iodinated casein on the concentration and synthesis of ubiquinone in the liver*

Weanling rats were fed with a diet containing 12% of casein and, where mentioned, 0.3% of iodinated casein for the specified experimental period. Ubiquinone synthesis was measured by the incorporation of radioactivity into liver ubiquinone from [2-¹⁴C]mevalonate (2 μ C/rat) 2 hr. after oral administration of the labelled compound. The values are the average of two independent determinations, i.e. on each of the two rats in a group.

Experimental period (days)	Supplements	Mean liver wt. (g.)	Concn. of ubiquinone (μ moles/g.)	Concn. of sterols (mg./g.)	Counts/min./liver			Specific radio-activity (counts/min./ μ mole of ubiquinone)
					Unsaponifiable lipids	Sterols	Ubiquinone (normalized)*	
5	Normal	2.8	142	1.46	31000	27180	350	880
	Iodinated casein	2.4	198	1.62	42660	37550	740	1560
10	Normal	3.4	70	1.20	52940	38960	260	1090
	Iodinated casein	2.8	150	1.52	54730	38650	590	1400
20	Normal	4.0	95	1.50	59930	43870	690	1820
	Iodinated casein	3.3	240	1.65	58140	41150	775	980

*Values of counts/min./liver in ubiquinone normalized to 100 000 counts/min. in total unsaponifiable lipids.

seen from the data (last two columns of Table 3) that the activation compared with appropriate normal controls was found at the 5 and 10 days stage but not at the 20 days stage. Also, the retention of radioactivity at 72 hr. after a single dose of the tracer was two- to three-fold that of normal animals (Table 4) in the intermediate stage of 10–20 days of feeding with iodinated casein, suggesting that 'catabolism' of ubiquinone was lowered.

Orally administered ubiquinone was absorbed and retained in the liver for longer periods in vitamin A-deficient rats (Joshi & Ramasarma, 1966) and rats exposed to cold (Aithal *et al.* 1968) than in normal animals and this was interpreted to be due to lowered catabolism. Repeating such an experiment with thyrotoxic rats revealed a peculiar feature. It is apparent from the data in Fig. 1 that the absorption of exogenous ubiquinone was less in thyrotoxic animals over short time-intervals.

Whereas in the normal animals retention of absorbed ubiquinone progressively decreased to low values at 96 hr., that in thyrotoxic animals increased. The radioactivity retained at this late time-interval was found mainly as ubiquinone and not as a degradation product. The orally dosed compound was normally excreted to a large extent and the intestines would be cleared of it within 48 hr. (Jayaraman, Joshi & Ramasarma, 1963). It is therefore surprising that such significant amounts of dosed ubiquinone were retained in the thyrotoxic animals either in the intestines or the blood, at the late time-interval before it reached the liver.

In summary, under hyperthyroid conditions, ubiquinone synthesis increased during early experimental periods. Simultaneously its catabolism in the liver was lowered, resulting in retention of the available ubiquinone. At later stages (after 10 days), the rate of synthesis declined to well below

Table 4. Retention of radioactivity in ubiquinone in the liver at 72 hr. after a single dose of [2-¹⁴C]mevalonate

Rats (two per group) were orally dosed with [2-¹⁴C]-mevalonate (2 μ C/rat) and killed after 72 hr. The animals during this period were fed on the same diet. The values are averages of the two independent determinations.

Experimental period (days)	Status	Ubiquinone (counts/min./liver)
5	Normal	750
	Fed with iodinated casein	950
10	Normal	360
	Fed with iodinated casein	880
20	Normal	540
	Fed with iodinated casein	880
34	Normal	200
	Fed with iodinated casein	760
20	Normal	200
	Thyroxine-supplemented (injection)	570

Fig. 1. Retention of radioactivity in the livers at different time-intervals after administration of a single dose of [¹⁴C]ubiquinone-10 (0.5 μ C/rat): ●, normal animal; ○, thyrotoxic animal.

normal, possibly as a consequence of the lowered catabolism of the ubiquinone already occupying the available functional sites in the liver. A similar situation seems to exist in two other conditions where hepatic accumulation of ubiquinone was observed: vitamin A deficiency (Joshi & Ramasarma, 1966) and exposure to cold (Aithal *et al.* 1968).

Intracellular distribution of the increased ubiquinone in the liver of thyrotoxic rats is shown in Table 5. The concentration in all the fractions increased but a major portion was in the mitochondrial and the supernatant fractions. The increase in the supernatant fraction (26% of the total) is in contrast with the low value reported by

Table 5. Intracellular distribution of ubiquinone in the livers of the thyrotoxic rats

The figures in parentheses are percentages in each fraction, taking the amount in the four fractions as 100%. The animals were fed with 12%-casein diet and, where mentioned, iodinated casein (0.3%) for 34 days. Each value represents the average of independent analyses from two rats.

Cell fraction	Ubiquinone (μ moles in cell fraction obtained from 1 g. of liver)		
	Normal	Thyrotoxic (fed with iodinated casein)	Thyrotoxic minus normal
Nuclear	15 (16)	47 (17)	32 (18)
Mitochondrial	53 (56)	125 (45)	72 (40)
Microsomal	19 (20)	33 (12)	14 (8)
Supernatant	8 (8)	71 (26)	63 (34)

Table 6. Effect of supplementary vitamin A on the increased liver ubiquinone in thyrotoxic rats

Weanling rats were fed with a diet containing 12% of casein and, where mentioned, 0.3% of iodinated casein for the specified period. In the supplemented animals 900 μ g. of vitamin A was orally administered per day per rat, in addition to the normal amount given to all the animals. The differences are significant ($P < 0.05$).

Experimental period (days)	Ubiquinone (μ moles/g. of liver)		
	Normal	Thyrotoxic (fed with iodinated casein)	Thyrotoxic with vitamin A supplement
5	100 $\pm$ 17	198 $\pm$ 18	175 $\pm$ 15
10	112 $\pm$ 16	248 $\pm$ 22	198 $\pm$ 16

Aiyar & Sreenivasan (1962) under somewhat milder conditions (feeding with 0.1% iodinated casein for 8 weeks) but is similar to that obtained in vitamin A deficiency (Heaton *et al.* 1957). Since it is known that vitamin A will be destroyed in thyrotoxic conditions (Sure & Buchanan, 1937), the effect of supplementing with extra vitamin A to compensate for the loss was tested and found to prevent the increase in liver ubiquinone to a small extent (Table 6).

Effect of giving supplementary thyroxine to rats on ubiquinone metabolism. In the second set of experiments, the effect of giving supplementary thyroxine, but at lower than toxic concentration, was tested. Administering thyroxine either orally or by subcutaneous injection resulted in increased concentration of ubiquinone in the liver, in agreement with the results of Edwin *et al.* (1960) and Brubacher & Wiss (1961), but not in other tissues.

Table 7. *Incorporation of [2-¹⁴C]mevalonate into unsaponifiable constituents in livers of rats given supplementary thyroxine and thiouracil*

(A) Weanling rats were fed with diets containing 12% of casein and, where mentioned, 0.3% of thiouracil for the specified experimental period. Thyroxine supplements were given as described in the Experimental section. (B) Male rats weighing about 160g. were fed with a diet containing 20% of casein and, where mentioned, 0.5% of thiouracil in the diet for 20 days. Ubiquinone-9 (1.5 mg./rat/day) was given orally for the last 10 days of the experiment. Ubiquinone synthesis was measured by the incorporation of radioactivity into liver ubiquinone from [2-¹⁴C]mevalonate (2 μ C/rat) 2 hr. after oral administration of the tracer. The values are the average of two independent determinations in each group.

					Counts/min./liver			Specific radio-activity (counts/min./ μmole of ubiquinone)	
Experi- mental period (days)	Supplements	Mean liver wt. (g.)	Concn. of ubiquinone (mμmoles/ g.)	Concn. of sterols (mg./g.)	Unsaponifi- able lipids	Sterols	Ubiqui- none (norma- lized)*		
(A)									
5	Normal	6.7	127	2.05	38520	28540	278	720	330
	Thyroxine (injection)	5.4	178	2.60	36280	26840	508	1400	530
	Thyroxine (dietary)	4.2	160	2.50	37830	29210	484	1280	720
10	Normal	6.1	128	2.60	56500	30000	310	550	400
	Thyroxine (injection)	5.4	214	2.41	21200	17000	480	2260	420
	Thyroxine (dietary)	4.4	220	2.77	33540	23340	360	1070	370
15	Normal	6.0	136	2.36	45750	33420	320	700	390
	Thyroxine (dietary)	5.7	210	2.33	25100	20400	276	1100	230
20	Normal	6.5	125	1.75	31680	22000	258	820	320
	Thyroxine (injection)	5.6	310	2.10	14650	12700	122	820	70
	Thyroxine (dietary)	6.1	260	2.20	15300	12900	112	720	70
	Thiouracil	5.9	110	2.11	40400	30650	190	470	290
	Thiouracil + thyroxine (injection)	5.7	214	2.33	46880	31000	670	1430	550
	Thiouracil + thyroxine (dietary)	6.3	221	1.64	36850	28700	470	1280	335
(B)									
20	Thiouracil	4.3	220	2.70	30500	23200	360	1180	380
	Thiouracil + feeding with ubi- quinone-9	5.5	510	2.14	26900	19700	200	750	70

*Values of counts/min./liver in ubiquinone normalized to 100000 counts/min. in total unsaponifiable lipids.

In such thyroxine-supplemented rats incorporation of radioactive mevalonate into liver ubiquinone increased, compared with the control animals at 5–10 days, and reverted to the low concentration at later periods (Table 7). The same picture was obtained with values for ubiquinone normalized to total radioactivity of 100000 counts/min. for unsaponifiable lipids and also on the basis of specific radioactivity. This comparison is especially valuable since the radioactivity in total unsaponifi-

able lipids decreased in thyroxine-supplemented animals at later experimental stages. Radioactivity retained at 72 hr. was higher (Table 4) in the supplemented animals, similar to thyrotoxicosis, indicating that catabolism was partially lowered as in thyrotoxicosis.

Effect of feeding with thiouracil on ubiquinone metabolism. In the third set of experiments, the effect of hypothyroid condition, as obtained by feeding with thiouracil for 20 days (Perelmutter,

Watanabe & Stephenson, 1965), was tested. The concentration of liver ubiquinone and incorporation of radioactive mevalonate into it decreased to a small extent (Table 7). Therefore the lack of thyroxine does not appear to influence the maintenance of ubiquinone concentration. Thyroxine-induced increase in liver ubiquinone concentration was obtained even in the presence of thiouracil. The effect of the presence of thiouracil and thyroxine combined gave some puzzling results. At the 20 days stage thyroxine supplements alone showed small decreases in the incorporation of mevalonate into ubiquinone, but addition of thiouracil, which by itself showed decrease, to the thyroxine-supplemented animals showed two- to three-fold increase in the synthesis of ubiquinone. This apparently is not due to any misleading increase in the mevalonate pool because the incorporation into 'total sterols' remained unchanged and also the same picture was obtained when values were normalized to 100 000 counts/min. for total unsaponifiable lipids. It appears that thiouracil reversed the inhibition by excess of thyroxine on the synthesis of isoprene compounds. In addition, it produced a condition of enhanced synthesis of ubiquinone in the presence of excess of thyroxine, an effect that neither of the supplements could produce individually at the 20 days stage. The presence of thiouracil reversed the action of excess of thyroxine at the 20 days stage to the pattern obtained at earlier stages (Table 7). Assuming that thyroxine-induced increase in the synthesis of ubiquinone existed even at late stages, this might be partially counteracted by the end-product inhibition believed to be operative at such high concentrations of ubiquinone present (Krishnaiah, Joshi & Ramasarma, 1967). One possible explanation of the thiouracil effect was considered to be a deletion of this end-product inhibition. This possibility was tested by feeding ubiquinone to thiouracil-supplemented rats, but it was found that the

inhibitory effect of excess of ubiquinone on the synthesis of ubiquinone from mevalonate was not abolished by thiouracil (Table 7).

Effect of addition of thyroxine in vitro on the synthesis of ubiquinone in liver slices. It was of interest to study whether the effects observed in intact animals were due to direct activation of synthesis of ubiquinone by thyroxine. Addition of L-thyroxine to the liver-slice system slightly inhibited the incorporation of mevalonate into ubiquinone (Table 8). The activation in the intermediate stage in the experiments *in vivo* was not found in the experiments *in vitro*.

Thyroxine status and isoprene synthesis. In the foregoing experiments on the changes in the incorporation of [2-¹⁴C]mevalonate into ubiquinone, it was important to evaluate whether the changes induced by altered thyroxine status are specifically on ubiquinone synthesis or on general isoprene synthesis. As a measure of the latter, the incorporation of radioactivity into total unsaponifiable lipids, constituting the majority of the isoprenoid lipid compounds, and into 'total sterols' fractions, were tested in the samples.

In early time-intervals (5–10 days) under hyperthyroid conditions, increased synthesis of isoprenoid compounds and sterols to the extent of 50% was observed but the increases in ubiquinone in the same samples were 200% or more. Therefore increased ubiquinone synthesis must be considered as an independent activation effect under hyperthyroid conditions. The decreases in synthesis at later time-intervals in thyroxine-supplemented animals (and strangely enough not with iodinated-casein feeding) and in thiouracil-fed animals are parallel for ubiquinone and 'sterols' and so represent a general effect on isoprene synthesis. The values obtained by normalizing the radioactivity in total unsaponifiable lipids to 100 000 counts/min. gave a similar but more precise picture of ubiquinone synthesis, not dependent on the changes in mevalo-

Table 8. *Effect of addition of thyroxine in vitro on the incorporation of [2-¹⁴C]mevalonate into ubiquinone by liver slices*

Preparation and incubation of liver slices were as described in the Experimental section. Values are obtained by analysis of three pooled samples in each set. Values in parentheses are the percentages recovered in the fraction, the total radioactivity in the unsaponifiable lipids being taken as 100%.

Addition	Counts/min./g. of liver			
	Unsaponifiable lipids	Sterols	Ubiquinone	Ubiquinone (normalized)*
Nil	46 800	33 800 (72)	170	360
L-Thyroxine	40 000	30 300 (76)	90	225

*Normalization: radioactivity in ubiquinone is calculated with the radioactivity in the unsaponifiable lipids taken as 100 000.

nate utilization, which was apparently altered probably because of the lowering of ATP known to occur under the conditions of excess of thyroxine (Fletcher & Myant, 1961).

DISCUSSION

In the experiments described in this paper, low-protein diets had been used to produce thyrotoxic conditions in the animals more rapidly. Under these conditions of protein insufficiency ubiquinone concentration in the tissues (Joshi, Jayaraman & Ramasarma, 1963b) and its synthesis in the liver (Joshi, Jayaraman & Ramasarma, 1965) were found to be lowered. Despite this, hyperthyroid conditions obtained either by feeding with iodinated casein or by supplementing with thyroxine led to the accumulation of ubiquinone in the liver, confirming many of the earlier observations. The present investigations demonstrated that the causes for this accumulation were enhanced synthesis and partly lowered catabolism of ubiquinone. The process required several days in the intact animals, which explains the lack of change in ubiquinone concentrations in the short-term experiments of Pedersen *et al.* (1963). Moreover, thyroxine in the normal animal apparently had no direct role in the maintenance of ubiquinone concentration or synthesis since lack of it (in thiouracil-fed animals) did not produce marked changes. Also, the influence of excess of thyroxine on ubiquinone metabolism appears to be indirect since addition of thyroxine *in vitro* to liver slices synthesizing ubiquinone did not elicit any activation effect, and in fact was inhibitory for isoprene synthesis. In view of this, thyroxine must be stimulating some other compound in the animal that in turn may activate ubiquinone synthesis. One possibility is adrenaline, known to be stimulated by thyroxine (Goodall, 1951; Hökfelt, 1951; Zile & Lardy, 1959). Adrenaline was shown to stimulate synthesis of ubiquinone in the liver of intact animals, but not in liver slices (A. R. Inamdar, unpublished work). Therefore both the hormones must be acting indirectly in the intact animal.

The increased ubiquinone in the liver of the hyperthyroid rat was found mostly in mitochondrial and supernatant fractions. Similar increase in mitochondrial ubiquinone was also found in cold-exposed rats (Aithal *et al.* 1968). The common factor in the two conditions is increased basal metabolic rate. Beyer *et al.* (1962b) proposed the hypothesis that increased ubiquinone may participate in calorogenic shunt pathways in the cell in cold exposure and in support of this concomitant increase was found in the ubiquinone-dependent succinate-neotetrazolium reductase

(H. N. Aithal, unpublished work) in addition to several other reductases and dehydrogenases. Therefore increased turnover of electrons by ubiquinone contributing in part to the 'increased' basal metabolic rate in hyperthyroid conditions seems possible.

In the experiments with incorporation of radioactive mevalonate, data on the total unsaponifiable lipids and sterols fraction were also collected. Examination of these data showed that the marked increase in the synthesis of ubiquinone was a specific response of hyperthyroid status of the animals and not a result of generalized effect on isoprene synthesis. The inverse relationship of thyrotoxic status and cholesterol had long been recognized (Peters & Van Slyke, 1946). However, there are reports that synthesis of cholesterol in the liver increased in hyperthyroid conditions (e.g. Byers, Rosenman, Friedman & Biggs, 1952; Fletcher & Myant, 1961) and that its catabolism increased even more accounting for the observed decrease in serum cholesterol concentration. Our data, limited as they are in representing the gross synthesis of isoprenoid compounds and particularly that of the mevalonate-sterol section of the pathway, are in support of the above hypothesis with respect to initial increase in isoprene synthesis.

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