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EFFECT OF COLD EXPOSURE ON THE METABOLISM OF UBIQUINONE IN THE RAT

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SUMMARY

1. Accumulation of ubiquinone in the livers of rats exposed to a cold environment was shown to be due to both decreased catabolism during the entire experimental period and increased synthesis during an intermediate stage (10–20 days).

2. The increased endogenous synthesis in the cold-exposed rats was eliminated when ubiquinone accumulated in the liver after exposure for 40 days (coinciding with acclimatization), or by absorption of the exogenous dietary supply, possibly by the mechanism of end-product regulation.

INTRODUCTION

Increased heat production necessary to maintain the body temperature of rats exposed to a cold environment was obtained in the first stage through shivering thermogenesis, primarily involving the peripheral regions of the body. On chronic exposure to cold, the animals adapt to the stress condition by increased food consumption followed by hypermetabolism wherein the abdominal core region seems to play an important role. POTTER[1] first postulated that, in prolonged cold exposure, calorigenic shunt pathways of electron transport of low phosphorylating efficiency would be activated to support the increased energy metabolism and heat production. Following this, a number of investigators have demonstrated increased activities of several liver oxidative enzymes in cold-exposed rats; *e.g.*, succinate dehydrogenase, cytochrome oxidase and malate dehydrogenase[2], microsomal NADH–cytochrome *c* reductase[3]. Also, BEYER, NOBLE AND HIRSCHFELD[4] have found a striking increase in the liver concentration of ubiquinone, known to participate in mitochondrial electron transport activities[5]. We present here our results showing that the increase in concentration of ubiquinone in the livers of cold-exposed rats was obtained through (a) an initially lowered catabolism which continued throughout the period of exposure and (b) an increased synthesis during a brief intermediate stage.

EXPERIMENTAL

Labelled compounds

DL-[2-^{14}C]Mevalonic acid lactone (2.37 mC/mmole) was obtained from the Radiochemical Centre, Amersham, England, and was delactonized before use. Sodium

[1-^{14}C]acetate (6.71 mC/mmole) was purchased from Bhabha Atomic Research Centre, Trombay. [^{14}C]Ubiquinone-10 (10 μC/mg) was a gift from Dr. O. WISS, Hoffmann-La Roche, Basle, Switzerland.

Animals and administration of tracers

Male albino rats from the institute colony weighing about 150 g were used throughout this study. The animals were exposed to cold in a refrigerated chamber at 0–5° for the stated number of days. They were fed a normal diet consisting of casein (20%), starch (60%), sugar (10%), peanut oil (5%), salt mixture (5%) and vitamins according to U.S.P. Water was given *ad libitum*.

The tracers, [2-^{14}C]mevalonate (2 μC/rat), [1-^{14}C]acetate (50 μC/rat) or [^{14}C]-ubiquinone-10 (0.5 μC/rat), were given orally and after the specified time interval the animals were killed by decapitation, the tissues were excised, cleaned of connective tissue and processed further.

In these experiments, 6 or more animals in each group were used. The results are expressed as mean values and standard deviation on the basis of wet tissue. In the radioactive experiments 2 animals were used in each group and the average values are given for the sake of brevity. The variation between independent determinations was normally within 10%.

Separation of ubiquinone and nonsaponifiable lipids

The tissues were saponified under reflux in ethanol (20 ml/g tissue) with aq. NaOH (40%, w/v) (1.0 ml/g tissue) in the presence of pyrogallol (0.1 g/g tissue) for 20 min. The nonsaponifiable 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, ethyl ether–light petroleum (5%, v/v) and ether–light petroleum (20%, v/v), respectively[6]. In the experiments with radioactive tracers, ubiquinone was further purified by thin-layer chromatography on Alumina G and then by repeated crystallization to constant specific radioactivity which usually remained unaltered after the thin-layer chromatography.

Preparation of cellular fractions

The livers were homogenized in 0.25 M sucrose (containing 0.01 M Tris (pH 7.4) and 0.005 M EDTA) in a Potter–Elvehjem-type glass homogenizer. Usually about 6–7 g of the liver samples were homogenized using 10 ml medium per g tissue and processed. The homogenate was separated into nuclear, mitochondrial, microsomal and supernatant fractions by the conventional differential centrifugation procedure of SCHNEIDER AND HOGEBOOM[7]. In these experiments 2 rats were used in each group and separately processed. The average values of two independent analyses are given in Tables II and III.

Determinations

Ubiquinone was measured by the decrease in absorption obtained on adding sodium borohydride to an ethanolic solution of the sample. Protein in the cell fractions was determined by the biuret method[8] using deoxycholate for solubilization. The radioactivity was measured using a Geiger counter (TGC 2; window thickness, 1.9 mg/cm^2) attached to a decade scaler (Nuclear Chicago Corporation Type 151A)

operated at 1500 V. The samples were plated as thin films on aluminium planchets and the counts obtained were corrected for self-absorption and background.

RESULTS

Increase in the concentration of ubiquinone in cold-exposed rats

Exposure of rats to low environmental temperature (0–5°) over a period of 40 days resulted in progressive increase in the concentration of ubiquinone in the liver (Fig. 1). In such animals, the liver weights had increased to the extent of 20 %

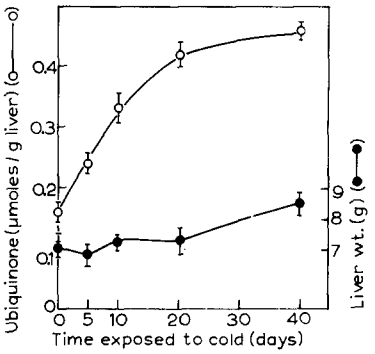


Fig. 1. Concentration of ubiquinone in the livers of rats exposed to cold. O—O, ubiquinone concentration; ●—●, liver weight.

TABLE I

CONCENTRATION OF UBIQUINONE IN INTESTINE AND KIDNEY IN COLD-EXPOSED RATS

Time exposed to cold (days)	*Ubiquinone concn. (mμmoles/g tissue)*	
	Intestine	*Kidney*
0	50 ± 12	61 ± 16
5	46 ± 14	73 ± 18
10	50 ± 8	87 ± 14
20	45 ± 10	77 ± 10
40	53 ± 13	93 ± 13

but the increase in ubiquinone was several-fold and was higher on the basis of both whole tissue and gram tissue, compared with the control animals. This marked increase was found only in the liver but not in the small intestine or kidney (Table I). Fractionation of the liver tissue by differential centrifugation of sucrose homogenates and analysing their ubiquinone content showed that the increased ubiquinone was distributed in all cell fractions, resembling the pattern in the normal animals[9], with a large portion being found in the mitochondrial and microsomal fractions (Table II).

Apparently the content of these cell components had not increased since the yields of protein in these fractions from equivalent amounts of the liver were the same in control and cold-exposed animals. Two marker enzymes, succinate oxidase in mitochondrial fraction and NADH–cytochrome *c* reductase in microsomal fraction,

showed only a 20–30 % increase under these conditions. But the concentration of ubiquinone in the liver and its cell components expressed on the basis of protein content showed several-fold increase in cold-exposed animals (Table II). Therefore, increased ubiquinone was not merely due to alteration in the content of mitochondria or microsomes but represents its accumulation in these fractions.

TABLE II

INTRACELLULAR DISTRIBUTION OF UBIQUINONE IN THE LIVERS OF COLD-EXPOSED RATS

The numbers of parentheses refer to percentage in each fraction taking the total in the 4 fractions as 100 %.

Cell fraction	*mμmoles ubiquinone in the cell fraction obtained from a g liver*		*mμmoles ubiquinone per mg protein*	
	Control	*Cold exposed (40 days)*	*Control*	*Cold exposed (40 days)*
Nuclear	28 (37)	52 (21)	0.6	1.3
Mitochondrial	31 (42)	94 (39)	2.1	6.4
Microsomal	10 (13)	70 (29)	0.2	1.7
Supernatant	6 (8)	26 (11)	0.1	0.4

Metabolism of liver ubiquinone in cold-exposed rats

Previous work in this laboratory showed that accumulation of liver ubiquinone in vitamin A deficiency was due to lowered catabolism and not to increased synthesis[10]. It was, therefore, of interest to study whether the accumulation of liver ubiquinone in cold exposure was due to a similar mechanism.

The synthesis and catabolism of ubiquinone in the liver was followed by the incorporation of [2-^{14}C]mevalonate at various time intervals after a single oral dose of the tracer. The time interval of 2 h was chosen to represent "synthesis" and that of 72 h, "catabolism", as described earlier[11]. If there was a lowering of catabolism of ubiquinone in cold-exposed rats, the radioactivity retained at 72 h, compared to the normal, would be higher. It is apparent that the initial response to cold exposure was to lower the catabolism of ubiquinone, as indicated by the higher amounts of radioactivity retained at 72 h. This persisted throughout and was more marked during the later periods of exposure to cold (Fig. 2); it seems to be responsible for the increase in ubiquinone in the early stage (up to 5 days) wherein the "synthesis" was not altered. On further exposure, at 10 days and after, "synthesis" increased 3- to 4-fold simultaneously with large increase in ubiquinone in the liver. When a sufficiently high concentration of liver ubiquinone was obtained at 40 days, by which time the animals were acclimatized to the cold[12], the rate of "synthesis" returned to the normal, low level.

The pattern of change in synthesis of ubiquinone in the liver, therefore, may be used as an index of the process of acclimatization of the rat to cold.

Absorption and retention of exogenous ubiquinone in cold-exposed rats

Earlier experiments in this laboratory demonstrated that orally given, radioactive ubiquinone-10 was absorbed and deposited exclusively in the liver, reaching a maximum at 24 h and subsequently decreasing, owing to catabolism[13]. If the catabolism was lowered it may be expected that the absorbed ubiquinone will be

retained in the livers for longer periods. The data in Fig. 3 support this hypothesis. The lowered catabolism was manifest all through the period of cold exposure, from 5 to 40 days.

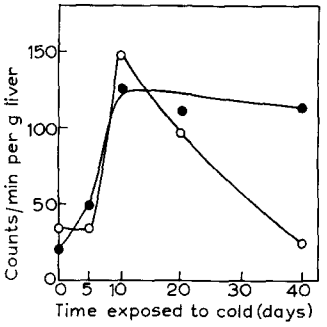


Fig. 2. Incorporation of [2-^{14}C]mevalonate (2 μC/rat) into liver ubiquinone of rats exposed to cold for different periods of time. Time intervals after dosing the tracer: O—O, 2 h; ●—●, 72 h.

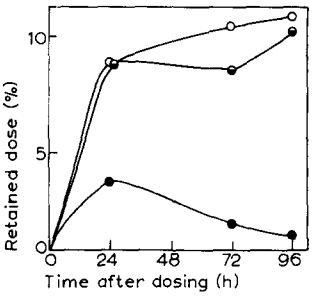


Fig. 3. Retention of radioactivity as per cent of dose present in the livers at different time intervals after administration of single dose of [^{14}C]ubiquinone-10 (0.5 μC/rat). Period of cold exposure: ●—●, unexposed; ◒—◒, 5 days; O—O, 40 days.

The exogenously supplied ubiquinone was distributed in all the cell fractions although the microsomal fraction had somewhat higher amounts, indicative of absorption of the exogenous lipid through the endoplasmic reticulum. In the cold-exposed animals, compared to the normal, there was a larger amount of radioactivity retained in each of the cell fractions and most of the increase was accounted for in the mitochondrial and microsomal fractions (Table III).

TABLE III

DISTRIBUTION OF ABSORBED [^{14}C]UBIQUINONE-10 IN THE LIVER CELL FRACTIONS OF COLD-EXPOSED RATS

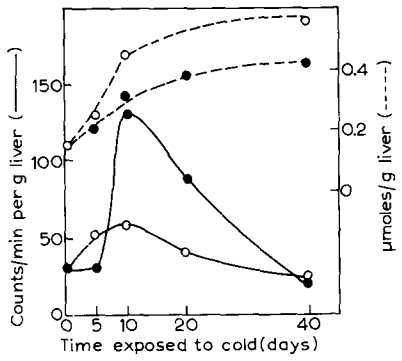
[^{14}C]Ubiquinone-10 (0.5 μC/rat) was orally administered to the rats which were killed after 48 h. The livers were processed as described in EXPERIMENTAL. In the normal and cold-exposed samples the 100% values correspond to 1100 and 2400 counts/min per liver, respectively.

Cell fraction	% *Distribution of the tracer*	
	Normal	*Cold exposed (40 days)*
Nuclear	16	7
Mitochondrial	58	43
Microsomal	25	39
Supernatant	10	11

Synthesis of liver ubiquinone in cold-exposed rats fed ubiquinone-9

It was demonstrated in this laboratory that feeding ubiquinone to rats increased its concentration in the liver and was accompanied by an inhibition of synthesis of both cholesterol and ubiquinone, at some step between acetate and mevalonate common in their synthesis, without affecting fatty acid synthesis[14]. A regulatory

function for ubiquinone in isoprene synthesis was proposed[15]. Since the "rate of synthesis", after an initial increase, was depressed during the late stages of cold exposure (see Fig. 2) simultaneously with increased liver-ubiquinone concentration, it was considered that this inhibitory effect might be by a mechanism similar to that obtained with dietary ubiquinone. It was therefore of interest to see whether the increased synthesis elicited by cold exposure could be prevented by increasing liver ubiquinone by supplying exogenous ubiquinone. The results in Fig. 4 show that feeding ubiquinone during cold exposure increased liver ubiquinone at a faster rate than cold exposure alone, and the pattern of increased incorporation of mevalonate at 10–20 days was nearly eliminated. Similar results were obtained with acetate as the tracer (Fig. 5).



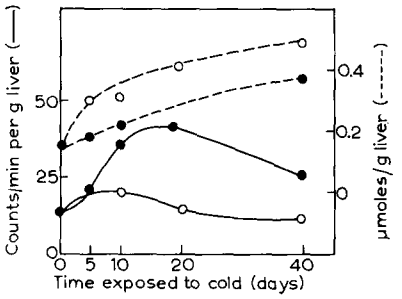


Fig. 4. Effect of feeding ubiquinone-9 (1 mg/day per rat) on the incorporation of [2-^{14}C]mevalonate (2 μC/rat) into liver ubiquinone of rats exposed to cold. O—O, ubiquinone fed; ●—●, normal; - - - -, ubiquinone concentration; ———, radioactivity incorporated at 2 h in ubiquinone.

Fig. 5. Effect of feeding ubiquinone-9 (1 mg/day per rat) on the incorporation of [1-^{14}C]acetate (50 μC/rat) into liver ubiquinone of rats exposed to cold. O—O, ubiquinone fed; ●—●, normal animals; - - - -, ubiquinone concentration; ———, radioactivity incorporated at 2 h in ubiquinone.

These results support the hypothesis that excess ubiquinone in the liver, obtained either by cold exposure or by direct feeding, inhibited endogenous synthesis of ubiquinone possibly by the mechanism of end-product inhibition.

DISCUSSION

Several theories have been proposed to explain increased heat production elicited to maintain the body temperature of cold-acclimatized rats, which had been reviewed by MASARO[16]. The basic mechanisms by which the energy is liberated as heat still remain to be established. Rapid transfer of electrons by way of calorigenic shunt pathways in mitochondria as well as microsomes appears to be the most likely means of achieving the production of excess heat[1,3]. To this end, components of such pathways have to be activated and increased in concentration.

The interesting hypothesis put forward by BEYER, NOBLE AND HIRSCHFELD[4] that increased ubiquinone during cold exposure may play such a role is worthy of consideration. The occurrence of ubiquinone in rat liver mitochondria[17] and microsomes[9] and the several-fold increase in concentration during cold exposure lend support

in favour of this view. The high concentration of ubiquinone in mitochondria (several-fold in molar excess of the other electron transfer components) and the observed, albeit controversial, low rate of its oxidation-reduction relative to the main coupled electron transfer chain have prompted the placement of ubiquinone in alternate shunt pathways[18,19]. Mitochondrial succinate–*neo*tetrazolium reductase is an example of a ubiquinone-dependent enzyme system[20] and its increased activity, found in cold-exposed animals simultaneously with increased concentration of ubiquinone, can explain rapid removal of electrons by this shunt pathway. It seems that excess heat production may be achieved by an integration of several such activated systems.

There are three stages in the alteration of ubiquinone metabolism in cold-exposed rats. In the first stage, at 5 days, there was lowered catabolism which continued throughout the cold exposure, conserving the normally synthesized ubiquinone. During the second stage, at 10–20 days, there appeared to be increased demand for ubiquinone which was met by increased synthesis *de novo*. In the third stage, at 40 days, when ubiquinone concentration was high, the rate of synthesis reverted to the normal level. The situation of lowered catabolism has been observed in other conditions such as vitamin A deficiency[10], experimental thyrotoxicosis[11] and essential fatty acid deficiency[21]. The common factor underlying these conditions seem to be stress on the animal. Invariably, the secondary response of increased synthesis followed in most of the stress conditions. It will be of interest to understand how these effects, characteristic only of the liver tissue in the rat, are employed by the animal as part of its defence mechanism against stress.

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