CONTENT OF UBIQUINONE-10 AND STEROLS IN THE LIVER OF HIBERNATING AND WAKING SUSLIKS

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Ubiquinone was isolated from the unsaponified fraction of liver lipids of the suslik (Citellus pygmaeus Pall.) by chromatography on a column of inactive alumina. The isolated ubiquinone was identified chromatographically (on a thin layer of Woehlm silica gel) and spectrophotometrically as ubiquinone-10. During the period of hibernation the ubiquinone concentration in the liver fell by more than 30%, and the content of total unsaponified sterols rose. The important role of ubiquinone in the regulation of heat production in hibernating animals is postulated.

An artificially induced increase in the ubiquinone concentration in the tissues is accompanied by an increase in the intensity of free nonphosphorylating oxidation. Ubiquinones evidently participate in the regulation of heat production in the body [6, 8, 9, 14]. The oxygen consumption in hibernating animals in a state of sleep is sharply reduced, their heat production increases, and their body temperature falls almost to the ambient temperature [1, 2]. One possible mechanism of the lowering of the body temperature in such animals is a decrease in the ubiquinone level in the organs.

The object of this investigation was to study the ubiquinone content in the liver of waking and hibernating susliks. Since the biosynthesis of ubiquinones in vivo is closely bound with biogenesis of the sterols [14], the opportunity was taken to investigate the content of total unsaponified sterols in the susliks' liver.

EXPERIMENTAL METHOD

Small susliks (Citellus pygmaeus Pall.) weighing 210±15 g were used. The waking animals were kept at a temperature of 15-20°C. They received cereals, vegetables, and dry hay for food. In the period of hibernation the susliks were kept at a temperature of 4-8°C for two to three weeks. The animals were decapitated, the liver was quickly removed, and ubiquinone was isolated [7], identified chromatographically on a thin layer of Woehlm silica gel and spectrophotometrically [13, 15], and estimated quantitatively, also spectrophotometrically [10]. Sterols were determined by their color reaction with acetic anhydride in concentrated sulfuric acid after their precipitation from the unsaponified fraction of lipids by digitonin solution. Protein was determined by the method of Lowry et al. [12]. The numerical results were subjected to statistical analysis [4].

EXPERIMENTAL RESULTS AND DISCUSSION

Several homologs of ubiquinone, differing in the length of their isoprenoid side chain [11], are found in animal tissues. The results of the investigations on a thin layer of silica gel showed that the unsaponified fraction of suslik liver lipids contains only one substance with chromatographic behavior similar to that of ubiquinone-10, isolated by the usual methods from the rat and guinea pig liver (Table 1). An alcoholic solution of ubiquinone from the suslik liver had the characteristic UV absorption spectrum of the ubiquinones [13]. The suslik liver thus contains ubiquinone-10.

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TABLE 1. Physical-Chemical Properties and Content of Ubiquinone Isolated from Liver of Waking and Hibernating Susliks

Source of ubiquinone	R_f in various systems of solvents				Fluorescence of spots, discs		Color of spots on moistening		Spectrophotometry of ethanol solution				Contents of ubiquinone (in μg; M ± m)		
	1	2	3	4	VL	UV	A	B	λ_{max}	λ_{min}	λ_{max}	λ_{min}	per gram wet weight	per gram protein	in the whole liver
Liver of waking susliks (n = 13)	0,29 ±0,02	0,52 ±0,02	0,72 ±0,03	0,42 ±0,02	Yel.	Dk.	Red	Blue	275	238	290	260	50,4 ±5,12	296,1 ±26,66	319,2 ±31,71
Liver of sleeping susliks (n = 10)	0,29 ±0,02	0,52 ±0,02	0,72 ±0,02	0,42 ±0,02	Yel.	Dk.	Red	Blue	275	238	290	260	27,5 ±5,22	170,8 ±18,69	176,7 ±28,75
Ubiquinone-9 from rat liver	0,29 ±0,02	0,52 ±0,02	0,72 ±0,02	0,52 ±0,02	Yel.	Dk.	Red	Blue	275	238	290	260	$P<0,05$	$P<0,05$	$P<0,05$
Ubiquinone-10 from rat liver	0,29 ±0,02	0,52 ±0,02	0,72 ±0,02	0,42 ±0,02	Yel.	Dk.	Red	Blue	275	238	290	260			

Legend: 1) Benzene, 2) chloroform : benzene (1 : 1); 3) 40% diethyl ether in hexane; 4) 95% aqueous acetone; thin layer of silica gel soaked with 5% petrolatum in petroleum ether; VL) visible light; UV) ultraviolet light; A) 0.5% solution of α,α'-dipyridyl in ethanol and 0.2% solution ferric chloride in ethanol mixed before use in the ratio 1 : 1, chromatograms treated first with saturated alcoholic solution of sodium borhydride; B) freshly prepared solution of reduced methylene blue (20 ml 1 mM methylene blue solution, 1 g powdered zinc, and 2 ml concentrated sulfuric acid) filtered through glass wall.

The mean content of ubiquinone-10 in the liver of the waking susliks was 50 μg/g body weight; during hibernation the ubiquinone content fell by more than 30%.

The ubiquinone concentration in the liver of mammals depends on thyroid activity: hyperthyroidism is accompanied by considerable accumulation of ubiquinone, but hypothyroidism by a decrease in its level and in the rate of its biosynthesis [10, 14]. During hibernation in susliks morphological evidence of inhibition of thyroid function [5] and a decrease in the blood level of thyroid hormones [3] are observed, and these could be connected with the decrease in the ubiquinone level found in the present experiments.

During hibernation the level of total unsaponified sterols in the susliks rose from 2.1±0.08 to 2.4± 0.12 mg/g wet weight of liver ($P < 0.05$). This is of considerable interest because the results of earlier investigations showed that the ubiquinone level and the sterol content in animal groups are inversely proportional to each other [14].

The results confirm the views expressed by other workers [6, 8, 14] regarding the role of ubiquinones in the regulation of heat production in mammals.

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