Pyridine Nucleotide-Cytochrome C Reductases in Rats Exposed to Low Oxygen Tensions. (25466)

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It has been shown that tissues from high altitude guinea pigs have a significantly higher succinoxidase system activity than those from sea level(1). However, "Antimycin titer" and cytochrome oxidase are little changed with altitude and the latter only in some tissues, indicating that the process of adaptation to high altitude may not necessarily involve modifications in all steps of a multienzyme system but rather in those which are rate-limiting factors for that system. DPNH and TPNH-cytochrome c reductases have been pointed out as very important steps in the respiratory chain, since the flow of electrons between, and through these 2 elements of the chain may be modified by a specific transhydrogenase in keeping with metabolic and energetic needs of the animal(2). All these facts prompted us to undertake a preliminary study concerning DPNH- and TPNH-cytochrome c reductases in liver of rats exposed to low oxygen tensions and compare the results with those obtained in sea level rats.

Methods. Two series of adult male albino rats were selected. Both groups were kept under the same standard conditions, except that rats for high altitude series were placed in low pressure chambers at a simulated altitude of 18,000 feet, for 23 hours daily, during 6 months. After killing animals by decapitation the liver was removed and a 10% homogenate was prepared in cold isotonic sucrose (0.25M). Cell fractions were obtained following the method of Schneider and Hogeboom(3). Enzymatic reduction of cytochrome c after addition of one of the reduced pyridine nucleotides was followed at 550 mμ in a model D. K. No. 2 Beckman Recording Spectrophotometer, at room temperature. The reaction mixtures and assay procedures were the same as those described previously(4). Specific activity is given as μM of cytochrome c reduced/minute/g of protein. The absorbancy index used for this reduction was 1.91×10^4 l mole^{-1} cm^{-1} (absorbancy index of reduced cytochrome c at 550 mμ-absorbancy index of oxidized cytochrome c at 550 mμ)(5).

Results. The liver from animals exposed to low oxygen tensions shows a higher DPNH-cytochrome c reductase activity than that from animals staying at sea level (Table I). Percentage of increment in activity of this enzyme is nearly the same in whole homogenate and in cell fractions. On the other hand, TPNH-cytochrome c reductase does not show any increment in animals exposed to high altitude; on the contrary, it tends to be lower than in the sea level group, although the difference has no statistical significance. To understand the results better, disregarding individual variations, ratios between DPNH- and TPNH-cytochrome c reductases were calculated for each animal, thus eliminating any variability due to changes in room temperature from one experimental day to another. Table II shows relative activities of these en-

TABLE I. Specific Activities of Pyridine Nucleotide-Cytochrome C Reductases in Liver from Sea Level and High Altitude Acclimated Rats.

	DPNH-cytochrome c reductase			TPNH-cytochrome c reductase		
	Sea level	High altitude		Sea level	High altitude	
	Means ± S.E. (8)*		P	Means ± S.E. (7)*		P
Whole homogenate	332.5 ± 22.5	463.1 ± 24.0	<.01	17.1 ± 5.4	14.6 ± 3.2	>1
Mitochondria	456.3 ± 29.3	717.8 ± 32.0	"	32.2 ± 6.4	29.4 ± 4.2	"
Microsomes	677.6 ± 67.4	1076.2 ± 85.6	"	26.3 ± 5.0	19.2 ± 3.7	"

* No. of animals. P = Probability based on t test of significance of differences between means.

TABLE II. Relative Activities of Cytochrome C Reductases in Rat Liver and Its Cell Fractions during Acclimatization to Simulated High Altitudes.

Tissue sample	Altitude	Cytochrome c reductases DPNH-	TPNH-
Whole homogenate	Sea level	19.4*	1
	18,000 ft	31.7	1
Mitochondria	Sea level	14.2	1
	18,000 ft	24.4	1
Microsomes	Sea level	25.7	1
	18,000 ft	56.0	1

* Figures are avg from 7 to 8 individual ratios.

zymes for each group of animals. In the altitude series the activity of the DPNH-cytochrome c reductase, with respect to the TPNH-system, is about twice as much as in the sea level control. It seems therefore, that during the process of adaptation to a low pressure environment there is a selective modification in transport of electrons toward oxygen. Both, DPNH- and TPNH-systems use oxygen as a terminal acceptor for electrons, though it is apparent that only the former generates high-energy phosphate bonds(2). These findings show an increase in activity of the high-energy generating system indicating that one of the features of acclimatization to a low oxygen tension atmosphere could be the improvement of those mechanisms involved in furnishing utilizable energy.

Summary. Pyridine nucleotide-cytochrome c reductase activities of whole rat liver and its cell fractions were determined in high altitude exposed rats and the results compared with those obtained in sea level rats. DPNH-cytochrome c reductase activity was higher in the low oxygen tension exposed rats, whereas the TPNH-cytochrome c reductase activity did not show any appreciable change. The significance of these findings is discussed.

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Pyridine Nucleotide-Cytochrome C Reductases in Acclimatization to Cold. (25467)

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Liver mitochondria from cold adapted hamsters have a higher succinoxidase and glutamic acid oxidase systems activity than those from control animals. Furthermore the succinic dehydrogenase and cytochrome oxidase activities are elevated in the former. To understand better the extent of enzymatic changes taking place during this adaptation, studies involving DPNH* and TPNH-cytochrome c reductases were undertaken.

Material and methods. Normal adult male hamsters kept in the cold (3° ± 2°C) for 70 days were killed by decapitation and their livers removed and placed in ice-cold isotonic sucrose. Mitochondria were prepared from a 0.25 M sucrose homogenate following the classic technic of Schneider and Hogeboom (1). Microsomes were obtained by centrifuging the mitochondrial supernatant at 105,000 x g for 50 minutes in Spinco preparative ultracentrifuge. Reduction of cytochrome c upon addition of one of the reduced pyridine nucleotides was recorded at 550 mμ in a model DK No. 2 Beckman spectrophotometer. Rates of reaction were measured at room tem-

* Abbreviations used: DPNH and TPNH = reduced diphospho and triphospopyridine nucleotides.