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.

TABLE I. Specific Activities of Pyridine Nucleotide-Cytochrome C Reductases in Liver-Cell Fractions from Control and Cold Exposed Hamsters.

		DPNH-cytochrome c reductase		TPNH-cytochrome c reductase	
		Mean ± S.E. (15)*	P	Mean ± S.E. (7)*	P
Mitochondria	Control	429.7 ± 29.4	>1	45.1 ± 4.0	>1
	Cold	466.5 ± 33.2		65.7 ±14.1	
Microsomes	Control	897.1 ± 54.8	< .02	15.3 ± 1.2	< .001
	Cold	1359.2 ±167.6		26.9 ± 2.3	

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

perature, and always one control and one cold exposed animal were studied experiment. Standard reaction mixtures and assay procedures have been described(2). Specific activity of samples was expressed in terms of μM of cytochrome c reduced/minute/g of protein at 550 mμ. Protein determinations were carried out by biuret reaction.

Results are presented in Table I. It may be noticed that the enzymatic activity of DPNH- and TPNH-cytochrome c reductases is not significantly changed in the mitochondrial liver fraction from cold exposed hamsters as compared with controls. Although the mean value of the TPNH-cytochrome c reductase is higher in the cold adapted animal, the difference has no statistical significance due to large individual variations. In the microsomal fraction, on the other hand, the activity of these enzymes is frankly elevated in the cold exposed group of animals. The data show an increment in activity of TPNH-cytochrome c reductase of about 77% over controls.

Discussion. It is of interest to consider the possible implications of these findings in the mechanism of cold acclimatization. Kaplan *et al.* showed that oxidation of TPNH is carried out with less generation of energy-rich phosphate bonds than the oxidation of DPNH (3). In turn, Vignais advanced the idea that oxidation of TPNH is accomplished with more heat evolution than oxidation of DPNH and that the increase in B.M.R. in the rat during Vit. A deprivation could be due to increment in oxidation of TPNH(4). Scholander *et al.*, on the other hand, have shown that in men acclimatized to cold, heat production remained much higher than the basal rate before acclimatization and that this increment is attained with no extra oxygen consumption(5). It is evident from these findings that the mechanism of cold acclimatization involves a metabolic adjustment resulting in increased heat production. It is also clear that oxidation of TPNH, as judged from this report, plays an important role in this acclimatization. Furthermore, the increment in activity of these reductases which occurs only in microsomes, and not in mitochondria which are regarded as the oxidative units in the cell, may signify that utilization of oxygen is not involved in the process as has been demonstrated in men by Scholander(5).

Summary. A study of the pyridine nucleotide-cytochrome c reductases activity was carried out in liver cell fractions (mitochondria and microsomes) from cold exposed hamsters. Control animals were studied simultaneously. Both DPNH- and TPNH-cytochrome c reductases had a higher activity in microsomes of cold adapted hamsters than in those of controls; the latter nevertheless increases more conspicuously. In the mitochondria, in turn, these 2 systems did not show significant changes. The possible implication of these findings in cold adaptation is discussed.

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