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SOME SPECIFIC EFFECTS OF HYPOBARIC HYPOXIA  
ON CELLULAR METABOLISM

BY

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US ARMY AEROMEDICAL RESEARCH LABORATORY

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reflect a metabolic control mechanism to maintain adequate energy production during periods of exposure to hypobaric hypoxic stress.

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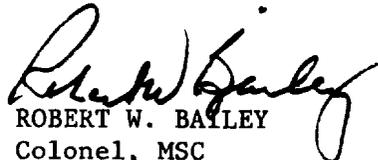
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US Army Medical Research and Development Command

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ABSTRACT

The US Army Aeromedical Research Laboratory is currently evaluating the problem of hypoxia in the Army aviation environment. Initial basic studies examined the effects of hypobaric hypoxia on the lactate and succinate dehydrogenase activities of hepatic tissue. After 6 and 12 hours incubation periods, the tissue samples exposed to an altitude of 36,000 feet (10,976 meters) demonstrated a significantly higher lactate dehydrogenase activity than did controls kept at ground level. In contrast, succinate dehydrogenase was significantly lower than controls after a 12 hour exposure to the experimental altitude. These changes in enzyme activity reflect a metabolic control mechanism attempting to maintain adequate energy production during periods of hypobaric hypoxic stress. These cellular metabolic derangements may be the cause of visual function decrement and altered mental proficiency which have been encountered in Army aircraft while flying at lower altitudes up to 10,000 feet (3,049 meters). The impact of prolonged flying at the relatively low altitudes of 10,000 feet (3,049 meters) or less has not been previously considered. Further study and correlation of this demonstrated altered metabolic state and the resultant performance decrement in unpressurized Army aircraft is necessary to insure combat mission completion.

  
ROBERT W. BAILEY  
Colonel, MSC  
Commanding

## INTRODUCTION

It is well known that many physiologic changes occur in animals during periods of exposure to high altitude. Over a period of time, some of these changes include hyperventilation, increased vascularity, and increased hemoglobin. These systemic compensatory reactions are known to aid man and other organisms in their survival during exposure to lowered oxygen partial pressures at altitude.

The physiologic and biochemical mechanisms that regulate the adaptation of animals to environmental stress like hypoxia and hypobaria are not clear at the present time. Exposure to hypobaric hypoxia could result in a lowered oxygen tension in the tissues, thus seriously affecting many metabolic processes which are dependent on molecular oxygen. The physiologic and biochemical responses to altitude favor the development of compensatory mechanisms to overcome the effects of the stress.

Several attempts have been made to determine if exposure to altitude results in changes in cellular metabolism. For example, the respiratory pigment, myoglobin, from both skeletal and cardiac muscle has been shown to increase during prolonged exposures to altitude (Anthony et al, 1959). There are also a number of reports on the effects of altitude exposure on tissue respiration. There is some controversy, however, concerning the findings of these reports. Some workers report tissue respiration is decreased during altitude exposure (Clark et al, 1954); others claim it is increased (Sundstroem and Michaels, 1942); while still others claim that it is unchanged (Frehn and Anthony, 1960).

The present study was undertaken in the hope of clarifying some of the contradictory findings concerning cellular metabolism during exposure to altitude. The specific aim of the study was to examine the effects of hypobaric hypoxia on two hepatic enzymes, lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH). By examining the activities of these two enzymes, it was possible to quantitate the effects of hypobaric hypoxia on both glycolytic and tricarboxylic acid cycle activity.

There are several problems encountered in deducing the effects of hypobaric hypoxia on the tissues of animals after in vivo exposures. One of these problems is the differential effects of hypobaric hypoxia on blood flow to different organs. For example, severe hypoxia produces a dramatic increase in coronary blood flow (Hackel et al, 1954) and a moderate increase in cerebral blood flow despite the associated hypocapnia (Lassen, 1959). It is clear that the actual degree of lowering of the intracellular oxygen tension cannot be predicted from most in vivo experiments. In light of this observation, it can be concluded that the study of the effects of hypobaric hypoxia on a particular tissue can only

be accomplished under conditions of complete ischemia or of controlled blood flow. By employing an in vitro approach in the present experiments, it was possible to circumvent the problem of perfusion changes and at the same time to quantitate the direct effects of hypobaric hypoxia on hepatic cellular metabolism.

Hypobaric hypoxia induced at altitudes as low as 10,000 feet is known to impair the function of a variety of tissues. Impaired visual function and decreased mental proficiency have been encountered in unpressurized Army aircraft without on-board oxygen equipment when flying at altitudes only slightly greater than 10,000 feet. It is currently believed that hypoxia-induced alterations of cellular metabolism may account for the impaired tissue function at even these operational altitudes. The rationale for our study was to determine if hypobaric hypoxia, independent of any cardiovascular alteration, could directly effect the activities of hepatic cellular enzymes. Follow-up studies will determine if the degree of hypobaric hypoxia which may be encountered in Army aviation has any detrimental effects on retinal cellular metabolism.

#### MATERIALS AND METHODS

Female adult Swiss Webster mice (approximate weight 35-40g) were used in all experiments. Mice were killed by cervical dislocation, and pieces of liver weighing approximately 150 mgs for the LDH assay or 450 mgs for the SDH assay were removed. The tissues were homogenized by a Polytron tissue homogenizer (Brinkman Instruments, Westbury, New York) after addition of 0.1 ml of phosphate buffer (0.034M, pH 7.4) to 1 mg of tissue for LDH and 5 mg of tissue for SDH, respectively.

The homogenate was centrifuged (Beckman model LZ-50 Ultracentrifuge) at 20,000 RPM for five minutes after which the supernatant was removed and placed on ice. For incubation, 100  $\mu$ l of the supernatant was added to each of 32 1-ml capacity incubation vials. Each incubation vial was tightly capped to prevent evaporation. The rubber middle of each cap was pierced by an 18 gauge hypodermic needle for the purpose of pressure equalization during the hypobaric treatments.

The incubation vials were divided into two groups, the controls and the experimentals. The control vials were placed in a desiccator containing filter paper dampened with water and were incubated at ambient barometric pressure. The experimentals were placed in a 9.3 liter capacity glass vacuum desiccator containing dampened filter paper. A Diaphragm Air Pump (model PV-200, Bell & Gossett-Leiman Bros., Monroe, LA) was used to create a vacuum equivalent to 23 inches of Hg (altitude equivalent, 36,000 ft) in the experimental desiccator. Both the control and experimental tissue samples were incubated for periods of 0, 6 or 12 hours. For the 6 and 12 hour incubations, the samples were maintained at a constant 4°C temperature.

Lactate dehydrogenase activities of the liver homogenates were determined by the method of Worthington (Worthington Biochemical Corporation, Freehold, NJ). Following incubation, the LDH activities of the homogenates were determined by measuring the spectral conversion of NADH<sub>2</sub> to NAD on a recording spectrophotometer (model 25, Beckman Instruments, Inc., Fullerton, CA) at a wavelength of 340 mμ at 20°C. The assay medium consisted of 2.7 ml phosphate buffer (0.034M, pH 7.4), 0.1 ml NADH<sub>2</sub> (0.0027M, pH 8.0) and 0.1 ml sodium pyruvate (0.01M, pH 7.0). At time zero 25 μls of the liver homogenate was added to the assay medium in a quartz cuvette and vigorously mixed. The changing optical density in the sample cuvette was compared to a blank (assay medium with 0.1 ml distilled water substituted for NADH<sub>2</sub>, plus 25 μls of tissue homogenate) for a 1.5 minute period.

The SDH activities were determined by measuring the spectral reduction of ferricyanide at a wavelength of 400 mμ (Kim and Han, 1969) at 20°C. The assay medium consisted of 0.3 ml of potassium cyanide (0.1M, pH 7.0), 0.3 ml potassium ferricyanide (0.01M), 0.4 ml of sodium succinate (0.5M), and 2.0 ml of Tris buffer (0.3M, pH 7.6). At zero time 25 μls of the liver homogenate was added to the assay medium in a quartz cuvette and thoroughly mixed. The changing optical density was compared to a blank containing the assay medium without the enzyme for a period of two minutes.

Protein determinations were done by a modification of the method of Lowry (Oyama and Eagle, 1956). Enzymatic activity is expressed in terms of μ moles of succinate or lactate converted/min/mg protein. Multiple comparisons of the means of enzyme activities were done by means of a Newman Keuls statistical test. Values considered significant have a p value of 0.05 or less.

## RESULTS

Recorded in Tables I and II are the mean changes in enzyme activity for LDH and SDH, respectively. Since there were no significant differences between the control and experimental tissue homogenates for the 0 hour incubation periods, it can be concluded that hypobaric hypoxia has no immediate effects on the enzymes. After 14 hours there was a significant decrement in enzymatic activity in both the control and experimental tissue homogenates; therefore, incubation periods were limited to 12 hours.

After a six-hour incubation period, the LDH activity of the experimental tissue homogenates was significantly higher than that of the controls and the same trend was maintained for the 12-hour incubations. In the case of SDH, both the control and experimental tissue homogenates after six hours showed an increased activity over those incubated for 0 hours. There was no significant difference in the SDH activities between tissues incubated for 12 hours and those incubated for 0 hours. After

six hours the SDH activity of the control homogenates was slightly higher than the experimentals. Between 6 and 12 hours there was a decrease in SDH activity in both the experimental and control tissue homogenates, and after 12-hour incubations the activities of the experimentals were also significantly lower than the controls. The experimental results for both LDH and SDH activities are graphically depicted in Figures I and II, respectively.

TABLE I

Mouse liver lactate dehydrogenase activity as influenced by hypobaric hypoxia. LDH activity determinations were made at a wavelength of 340 m $\mu$  at 20°C and are expressed in terms of  $\mu$ m of enzyme/min/mg protein.

INCUBATION PERIOD (hours)	CONTROL (P <sub>O</sub> <sub>2</sub> =158.5mmHg) Mean $\pm$ SE (N)	EXPERIMENTAL (P <sub>O</sub> <sub>2</sub> =35.9mmHg) Mean $\pm$ SE (N)	%CHANGE
0	10.31 $\pm$ 1.52(47)	10.27 $\pm$ 1.50(48)	-0.4
6	9.14 $\pm$ 0.99(86)	9.92 $\pm$ 1.08(35)*	+7.9
12	8.90 $\pm$ 1.31(47)	9.40 $\pm$ 1.39(47)*	+5.4

\* Significant difference in LDH activity at the 5% level.

TABLE II

Mouse liver succinate dehydrogenase activity as influenced by hypobaric hypoxia. SDH activity determinations were made at a wavelength of 400 m $\mu$  at 20°C and are expressed in terms of  $\mu$ m of enzyme/min/mg protein.

INCUBATION PERIOD (hours)	CONTROL (P <sub>O</sub> <sub>2</sub> =158.6mmHg) Mean $\pm$ SE (N)	EXPERIMENTAL (P <sub>O</sub> <sub>2</sub> =36.0mmHg) Mean $\pm$ SE (N)	%CHANGE
0	0.4567 $\pm$ 0.0681(46)	0.4472 $\pm$ 0.0666(46)	-2.1
6	0.5186 $\pm$ 0.0615(72)	0.4920 $\pm$ 0.0584(72)	-5.2
12	0.4484 $\pm$ 0.0654(48)	0.3891 $\pm$ 0.0574(47)*	-13.3

\*Significant difference in SDH activity at the 5% level.

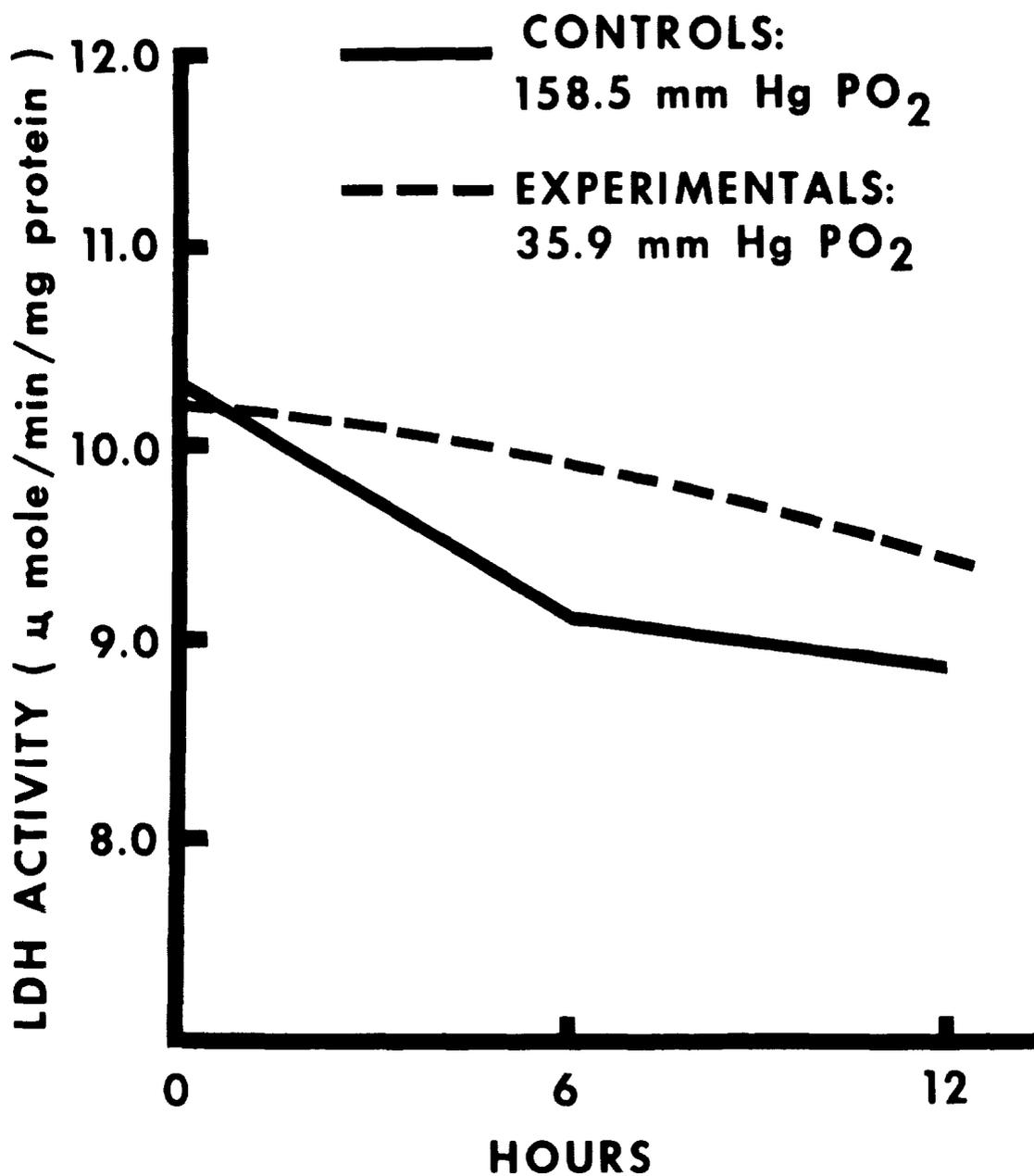


FIGURE I

Lactate dehydrogenase activity ( $\mu$  moles/min/mg protein) of mouse liver homogenate as influenced by hypobaric hypoxia (35.9 mm Hg PO<sub>2</sub>) or normobaric oxygen tensions (158.5 mm Hg PO<sub>2</sub>) at 20°C. Plotted are the means. Refer to Table I for statistical details.

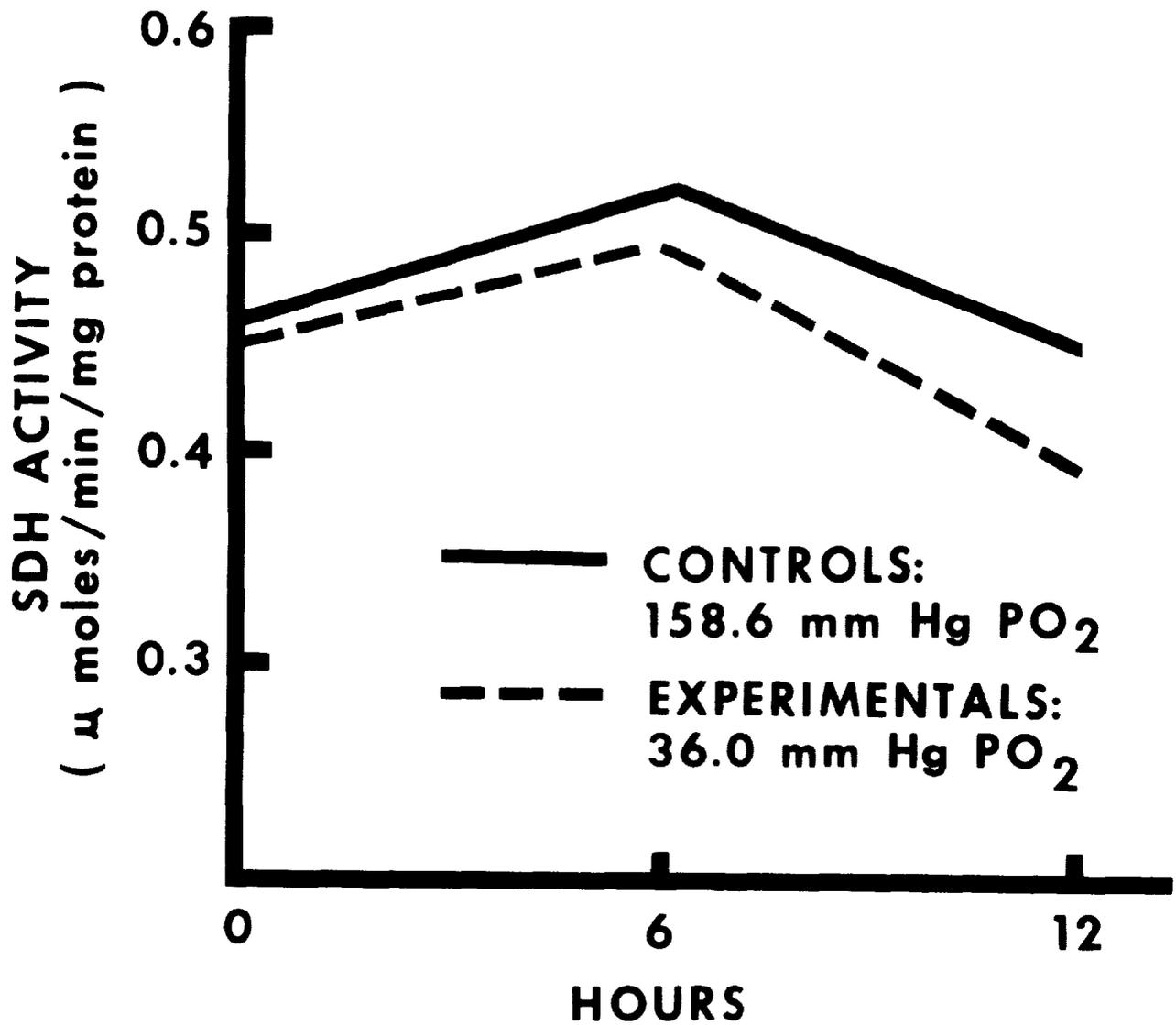


FIGURE II

Succinate dehydrogenase activity ( $\mu$  moles/min/mg protein) of mouse liver homogenate as influenced by hypobaric hypoxia (36.0 mm Hg  $P_{O_2}$ ) or normobaric oxygen tensions (158.6 mm Hg  $P_{O_2}$ ) at 20°C. Plotted are the means. Refer to Table II for statistical details.

## DISCUSSION

The specific increase in SDH activity in both experimental and control liver tissue homogenates after 6 hour incubation periods may be considered to be of physiological significance to the animal. In the case of the experimental homogenates, the available oxygen to the cells is decreased. In a 6 hour exposure to such conditions, the enzyme system might have to be regulated to provide adequate energy requirements to the cell. The limiting enzyme in the sequence of electron transport is SDH, therefore, this is the obvious site for a change to increase the overall rate despite the lowered oxygen concentration. In this context the modulation of the rate limiting SDH activity to increase the overall rate of oxidation of succinate would become meaningful to maintain the energy requirements of the cell. The increase in the SDH activity of the control tissues after 6 hours is more difficult to explain. The increased activity is not likely a response to hypoxia, but is probably a metabolic response to the unavoidable stress of the in vitro situation.

The fact that SDH activity decreases after exposures to hypobaric hypoxia as compared to controls exposed to normobaric oxygen tensions suggests that other mechanisms may be occurring. In a study by Aschenbrenner et al (1971) in which mice were exposed to 4-5% O<sub>2</sub> for 6 hour periods, it was found that there was a significant decrease in cardiac muscle mitochondria. It was concluded that tissue oxygen tension is a potent regulator of mitochondrial functional mass in mammalian cardiac muscle. The reduction in hepatic SDH activity after exposure to hypobaric hypoxia in the present experiments may reflect a similar reduction in functional hepatic mitochondria.

Bartley et al (1968) observed that during periods of oxygen deficiency there is a decrease in phospholipid synthesis for mitochondrial membrane formation. Phospholipids have specific effects on the catalytic efficiency of certain enzymes. Phospholipids are necessary not only for the succinoxidase system and various fragments of the respiratory chain but for the succinic flavoprotein as well (Hafkenschied et al, 1963). It seems reasonable to suppose that the effect of phospholipids on SDH occurs at the level of the enzyme molecule either by producing a favorable medium for the reaction or by modifying the protein (Cerletti et al, 1965). The decrease in SDH activity on exposure to altitude in our experiments may be the result of a decrease in cellular phospholipids.

There have been numerous studies attempting to elucidate the effects of hypobaric hypoxia on mitochondrial function. After a continuous exposure of rats to a simulated altitude of 25,000 feet for several days, it was observed that there were significant decreases in the respiratory capacity of liver and kidney mitochondria (Gold et al, 1973). Strickland

et al (1962) observed a decline in the respiration of liver mitochondria from rats exposed to 21,000 feet for 6-7 weeks. Nelson et al (1967) found a decrease of 15% in the same parameter of rats exposed to 25,000 feet for 3 days. On the basis of these collective in vivo findings, it appears that mitochondrial respiratory capacity, at least in the liver, falls relatively early in chronic altitude stress and remains below normal. The present in vitro study has shown that an exposure as short as 12 hours to an altitude of 36,000 feet can result in a significant decrease in hepatic SDH activity. This hypobaric hypoxic induced decrease in SDH activity, if not compensated for by some other mechanism, would similarly result in a decreased respiratory activity of the hepatic cells.

Acute hypoxemia has been found to produce oxygen debt, raise the lactate to pyruvate ratio and result in the accumulation of excess lactate in the blood of several different species of experimental animals including man (Huckabee, 1965 and Gold et al, 1973). In our experiments there was a significant increase in LDH activity after exposure to hypobaric hypoxia for 6 and 12 hours. This increase in LDH activity coupled with the decrease in SDH activity of the experimental tissue homogenates may be part of a cellular control mechanism to maintain metabolic efficiency during hypobaric hypoxic stress. Although the respiratory capacity of the mitochondria is altered, there may not be a decrease in total energy production by the cell. The increase in LDH activity intimates an increase in the glycolytic rate of the cell. Thus there is a metabolic compensatory mechanism in an attempt to maintain adequate energy production during periods of hypobaric hypoxia.

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