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ABSTRACT

The metabolic effects of hypobaric hypoxic stress on the mammalian liver were studied. The lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH) activity of mouse liver homogenates were measured after exposure to an equivalent altitude of 36,000 feet and compared to controls kept at zero altitude. After six and twelve hour incubation periods, the altitude exposed samples demonstrated a significantly higher LDH activity than controls. SDH activity remained unchanged from controls after six hours but was significantly lower than controls after a 12 hour exposure to altitude. It is concluded that the changes in enzyme activity reflect a metabolic control mechanism attempting to maintain adequate energy production during periods of exposure to hypobaric hypoxic stress.

INTRODUCTION

It is well known that many physiological changes occur in animals during periods of exposure to high altitudes. Over a period of time, some of these changes include hyperventilation, increased vascularity, and increased hemoglobin. These changes can be considered compensatory reactions and are known to aid in the survival of man and other organisms during periods of exposure to lowered oxygen partial pressure at altitude, a condition known as hypobaric hypoxia.

The biochemical mechanisms that regulate the adaptation of animals to environmental stress like hypobaric hypoxia are not clear at the present time. Exposure to hypobaric hypoxia could result in a lowered oxygen tension in the tissues, thus, seriously affecting those metabolic processes which are dependent on molecular oxygen. The biochemical and physiological responses to altitude exposure should 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 oxygen storage pigment, myoglobin, from both cardiac and skeletal muscle has been shown to increase during prolonged exposure 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 have reported that tissue respiration is decreased during altitude exposure (Clark et al, 1954); others have claimed that it is increased (Sundstroem and Michaels, 1942); while still others have claimed that it is unchanged (Frehn and Anthony, 1961).

Our study was undertaken in the hope of clarifying some of the contradictory findings concerning cellular metabolism during exposure to altitude. The specific aim was to examine the effects of hypobaric hypoxia on two hepatic enzymes in the mouse: 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 the activity of both the Embden-Meyerhof pathway and the tricarboxylic acid cycle.

There are several problems encountered in trying to deduce the effects of hypobaric hypoxia on the tissues of animals after *in vivo* exposures to altitude. A particularly important problem is the different effect of hypobaric hypoxia on the blood flow to different organs. For example, severe hypobaric hypoxia results in a dramatic increase in coronary blood flow (Hackel et al, 1954) but only a moderate increase in cerebral blood flow (Lassen, 1959). It is clear that, due to these differences in perfusion, the actual degree of lowering of the intracellular oxygen tension cannot be predicted from most *in vivo* experiments. In light of this observation, 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 our study 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.

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 mg for the LDH assay or 450 mg 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 μl 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 $NADH_2$ 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 $NADH_2$ (0.0027M, pH 8.0) and 0.1 ml sodium pyruvate (0.01M, pH 7.0). At time zero 25 μl 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 $NADH_2$, plus 25 μl 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 times 25 μl 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 6-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 6 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 6 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 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 mμ at 20°C and are expressed in terms of μm of enzyme/min/mg protein

INCUBATION PERIOD (hours)	CONTROL (P_{O_2}=158.5mmHg) Mean ± SE (N)	EXPERIMENTAL (P_{O_2}=35.9mmHg) Mean ± SE (N)	%CHANGE
0	10.31 ± 1.52 (47)	10.27 ± 1.50 (48)	-0.4
6	9.14 ± 0.99 (86)	9.92 ± 1.08 (85)*	+7.9
12	8.90 ± 1.31 (47)	9.40 ± 1.39 (47)*	+5.4

*Significant difference in SDH 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 mμ at 20°C and are expressed in terms of μm of enzyme/min/mg protein.

INCUBATION PERIOD (hours)	CONTROL (P_{O_2}=158.6mmHg) Mean ± SE (N)	EXPERIMENTAL (P_{O_2}=36.0mmHg) Mean ± SE (N)	%CHANGE
0	0.4567 ± 0.0681 (46)	0.4472 ± 0.0666 (46)	-2.1
6	0.5186 ± 0.0615 (72)	0.4920 ± 0.0584 (72)	-5.2
12	0.4484 ± 0.0654 (48)	0.3891 ± 0.0574 (47)*	-13.3

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

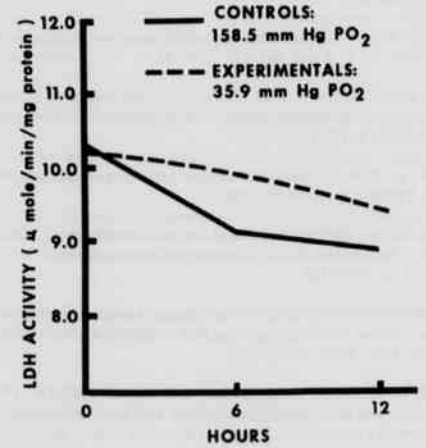

Figure 1. Lactate dehydrogenase activity (μ moles/min/mg protein) of mouse liver homogenate as influenced by hypobaric hypoxia (35.9 mmHg P_{O_2}) and normobaric oxygen tensions (158.5 mmHg P_{O_2}) at 20°C. Plotted are the means. Refer to Table I for statistical details.

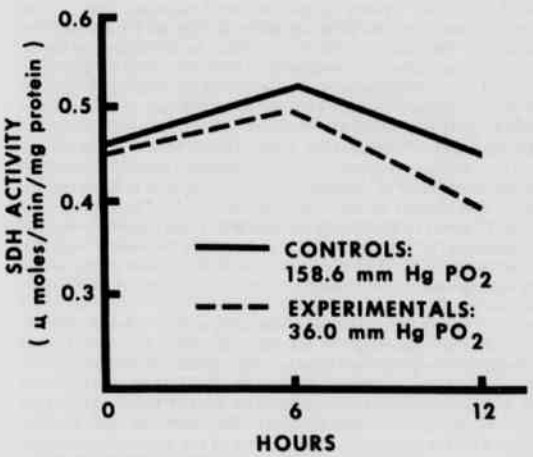

Figure II. Succinate dehydrogenase activity (μ moles/min/mg protein) of mouse liver homogenate as influenced by hypobaric hypoxia (36.0 mmHg P_{O_2}) and normobaric oxygen tensions (158.6 mmHg P_{O_2}) at 20°C. Plotted are the means. Refer to Table II for statistical details.

DISCUSSION

The specific increase in SDH activity in both the experimental and control liver tissue homogenates may be of physiological significance. In the case of the experimental homogenates, the available oxygen is decreased. In a 6 hour exposure to such conditions the enzyme systems might have to be regulated to meet the energy requirements of the cell. In the mammalian liver 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 of cellular respiration 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 homogenates after 6 hours is more difficult to explain. The increased activity is not likely a response to hypoxic stress, 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% 0_2 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 a 12 hour exposure to hypobaric hypoxia in our 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 not only necessary for the activity of the succinoxidase system and various fragments of the electron transport chain but they are also necessary to maintain the activity of SDH (Hafkenscheid et al., 1963). 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 upon exposure to altitude in our experiments may be the result of a decrease in 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, there were significant decreases in the respiratory capacity of liver and kidney mitochondria (Gold et al., 1973). In addition, Strickland et al. (1962) observed a decline in the respiration rate 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 in rats exposed to 25,000 feet for 3 days. On the basis of these collective *in vivo* findings, it appears that mitochondrial respiratory activity 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 homogenates.

Hypoxia 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 increased LDH activity coupled with the decreased 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 lowered, there may not be a decrease in the total energy production of the cell. The increase in LDH activity intimates an increase in the glycolytic rate of the cell. Thus, there is a metabolic compensatory mechanism at work attempting to maintain adequate energy production during exposure to hypobaric hypoxia.

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