Effect of High Altitude on Lactic Dehydrogenase Isozymes and Anoxic Tolerance of the Rat Myocardium (35915)

G. L. Anderson and R. W. Bullard
(Introduced by X. J. Musacchia)

Space Sciences Research Center, University of Missouri, Columbia, Missouri 65201; and Department of Anatomy and Physiology, Indiana University, Bloomington, Indiana 47401

The LDH isozyme complement in various organs has been related to their oxygen consumption. In an organ such as the heart, which is capable of a highly aerobic metabolism there is a predominance of H-subunits, while in organs in which the energy demand is satisfied by glycolysis the M-type subunit predominates (1). The observation that the subunits exist in varying proportions in mammalian tissues and that the distribution is related to the oxygen consumption of the tissue has lead to the suggestion that isozymes of LDH have different physiological functions (2).

As a consequence of the proposed functional roles for the isozymes of LDH a number of studies have been carried out to determine if the LDH complement of a tissue is altered in response to changes in oxygen availability. Myocardial tissue has received a good deal of attention. The LDH complement of monkey heart cells in tissue culture is altered by changing the oxygen tension (3). Furthermore, chronic exposure to the hypoxia of altitude may significantly alter the H/M ratio in cardiac tissue of adult rats (2), though such an effect is not universally agreed upon (4).

In this study, changes in isozyme complement, as well as the anoxic tolerance of the heart, in response to the hypoxia of altitude have been investigated. The question as to whether shifts in LDH complement are of any physiological advantage is discussed.

Materials and Methods. Male albino rats, weighing from 200 to 350 g, were chosen for these experiments. They were housed in animal quarters in which the temperature was maintained at 22 ± 1°, and fed a diet of Purina rat chow and tap water *ad libitum*.

Acclimation was carried out in chambers in which the ambient pressure could be reduced. The acclimation procedure was divided into two phases; an ascent phase and a continual exposure phase. In the ascent phase, animals were exposed for 1 day each at 10,000 feet and 12,500 feet, and for 2 days each at 15,000 feet and 18,000 feet. In the continual exposure phase animals were acclimated to 22,500 feet for 15 days. Exposure was interrupted for 4 hr out of each 76 hr period so that litter could be changed and fresh food provided.

Following acclimation, that is after the 15-day exposure to 22,500 feet, the animals were sacrificed and the whole heart was rapidly excised. The heart was prepared for evaluation of LDH complement or resistance to experimental anoxia.

Homogenates of whole heart were prepared using 0.1 *M* Tris buffer at pH 8.2 as the extraction medium. A supernatant fraction separated at 15,000–20,000*g* for 15 min was tested for the isozymes of LDH.

Starch gel electrophoresis was used to separate the isozymes of LDH. The stained gels were read on a recording densitometer and the results were quantified by measurement of the area described by the densitometer tracing of each LDH band. The percentage of H-subunits in the total LDH complement of the tissue was calculated (5).

The anoxic tolerance of the heart was tested using the isolated right ventricle (6). Both the time required to reduce contractile strength to one half of its preanoxic value (7), designated $T_{1/2}$, and the percentage recovery following anoxia (8) were determined.

Results. Five isozymes of LDH were de-

TABLE I. $T_{1/2}$, Percentage Recovery, Percentage H-Subunits, and H/M Ratio for Myocardium of Sea Level and Altitude-Acclimated Rats.

	No. of animals	Mean ± SD	Significance ($p < .025$)[a]
$T_{1/2}$ (min)			
Control	9	3.9 ± 1.0	NS
Acclimated	14	4.1 ± 1.1	
Recovery[b] (%)			
Control	7	65.0 ± 20.1	S
Acclimated	13	83.8 ± 11.4	
H-Subunits (%)			
Control	8	68.5 ± 4.1	S
Acclimated	7	62.1 ± 5.2	
H/M ratio			
Control		2.17	—
Acclimated		1.67	

[a] One tailed t test accepting the .025 level of significance.

[b] Percentage recovery was determined at 20 min into the postanoxic period.

[c] No test of significance applied.

tected in the myocardium of both nonacclimated and altitude acclimated rats. The percentage of the total LDH complement in each of the isozyme forms was estimated in eight nonacclimated and seven acclimated hearts. The percentage of the total LDH present as the H_4 isozyme was significantly reduced ($p<.001$) in the acclimated hearts. In addition, the percentage of H-subunits in the total LDH complement dropped from 68.5% in nonacclimated hearts to 62.1% in hearts isolated from acclimated animals (Table I). This difference was significant at the .025 level. There was no significant difference in the total LDH complement of the acclimated and nonacclimated hearts.

The myocardium of rats acclimated to altitude appears to be more resistant to anoxia than that of nonacclimated rats. Though the $T_{1/2}$ value determined for acclimated ventricles, 4.1 min, was not significantly longer than that of controls, 3.9 min, the acclimated tissues were able to recover more completely from an anoxic exposure than the nonacclimated ventricles. The percentage recovery determined for acclimated ventricles, 83.8%, was significantly greater ($p<.025$) than that determined for controls, 65.0% (Table I).

Discussion. Animals exhibit a variety of physiological adjustments when exposed chronically to the hypoxia of altitude (9). There is some evidence that anaerobic metabolism of the myocardium is enhanced by exposure to altitude (10). In this study, we examined the LDH complement of the myocardium following altitude acclimation. In addition, an attempt was made to determine whether shifts in LDH isozymes are of any physiological advantage to the animal.

Evaluation of the myocardium LDH complement revealed a shift in response to the hypoxia of altitude. The H/M ratios calculated from the data reported are 2.17 for control ventricles and 1.66 for altitude acclimated ventricles. These values are in good agreement with those in an earlier report (2) in which the H/M was reduced from 2.20 to 1.85 following 45 days exposure to 14,500 feet. The conflicting results reported by Miller and Hale (4) do not appear to be explainable even on the basis of the degree of the hypoxic stress imposed.

Furthermore, ventricles isolated from the altitude-acclimated animals proved to be more resistant to experimental anoxia than those isolated from nonacclimated controls. The functional adaptation reported here is of the same order of magnitude as that observed by other investigators (8) using the same stimulation frequency, *i.e.*, 60/min.

We conclude that there are parallel changes in LDH complement and anoxic tolerance of the myocardium and suggest that the observed shift in the LDH may contribute to the increased anoxic tolerance of the altitude-acclimated myocardium. However, due to the multitude of factors which effect contractility of the isolated ventricle this hypothesis must as yet be viewed as equivocal.

Summary. Exposure of rats to the chronic hypoxia of altitude has two demonstrable effects upon the myocardium. (i) There is an alteration of the LDH complement of the tissue such that the H/M ratio is reduced. (ii) The ability of the myocardium to recover function following an anoxic stress is enhanced. The results are consistent with the view that shifts in tissue complement of

LDH isozymes are adaptive.

1. Dawson, D. M., Goodfriend, T. L., and Kaplan, N. O., Science **143**, 929 (1964).

2. Mager, M., Blatt, W. F., Natale, P. J., and Blatteis, C. M., Amer. J. Physiol. **215**, 8 (1968).

3. Kaplan, N. O., J. Cell. Comp. Physiol. **66**, (Suppl. 1), 1 (1968).

4. Miller, A. T., Jr., and Hale, D. M., J. Appl. Physiol. **25**, 725 (1968).

5. Hellung-Larsen, P., and Andersen, V., Exp. Cell. Res. **80**, 286 (1968).

6. Poupa, O., Korecky, B., Krofta, K., Rakusan, K., and Procházka, J., Physiol. Bohemoslov. **13**, 281 (1964).

7. McGrath, J., Bullard, R. W., and Komives, G. K., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **28**, 1307 (1969).

8. Krofta, K., Procházka, J., and Poupa, O., Physiol. Bohemoslov. **14**, 238 (1965).

9. Barbashova, Z. I., "Handbook of Physiology," Sect. 4, pp. 37. Amer. Physiol. Soc., Washington, DC (1964).

10. Burlington, R. F., Whitten, B. K., and Bowers, W. D., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **29**, 853 (1970) Abstr. only.

Received June 9, 1971. P.S.E.B.M., 1971, Vol. 138.