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MEMORANDUM REPORT NO. 68-6

CARBONIC ANHYDRASE ANALYSES OF THE BLOOD OF SUBJECTS EXPOSED TO A HELIUM-OXYGEN ENVIRONMENT AT SEVEN ATMOSPHERES PRESSURE

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SUMMARY PAGE

THE PROBLEM

To measure the enzyme system, carbonic anhydrase, which is primarily responsible for controlling tissue carbon dioxide tension, in the blood of a group of men isolated in a helium-oxygen atmosphere under seven atmospheres of pressure.

FINDINGS

Comparison of the control activity values for carbonic anhydrase with those obtained after exposure to the experimental environment indicates that the potential enzymic activity is not markedly altered under these conditions.

APPLICATIONS

This information contributes to the basic knowledge of the regulatory mechanism of gaseous components involved in normal metabolism, which is of prime importance to the health of men working under pressure.

ADMINISTRATIVE INFORMATION

This investigation was conducted as a part of Bureau of Medicine and Surgery Research Work Unit MR005.04-0053.03—Enzymatic Responses to Environmental Challenges. The present report is No. 3 on this Work Unit. It was approved for publication on 3 April 1968 and designated as Memorandum Report No. 68-6.

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INTRODUCTION

Regulation within the tissues of gaseous components involved in normal metablism is of obvious importance to the health of men working under pressure. The activity of the enzyme system, carbonic anhydrase, primarily responsible for controlling tissue carbon dioxide tension, was therefore measured in the blood of three men being studied under seven atmospheres of pressure in a helium-oxygen environment.*

METHODS OF EVALUATION

Enzymic activity determinations were carried out at 25°C on diluted whole blood hemolysates by a modified Philpot and Philpot procedure (1,2) over the pH range 8.6 to 7.6 employing phenol red as the end point indicator. The reaction was buffered with 0.15 M tris(hydroxymethyl)aminomethane and the substrate, CO_2, was added to the mixture as a regulated stream of gas bubbles. Activity values are reported for hemolyzed whole blood after dilution as indicated.

Electrophoretic analyses were performed employing polyacrylamide gel columns according to the method of Davis (2). The semi-purified enzyme solutions analyzed electrophoretically were obtained by an extraction with chloroform-ethanol of erythrocytes triply washed with isotonic saline and hemolyzed in distilled water (3).

OBSERVATIONS AND DISCUSSION

A comparison of the control activity values for carbonic anhydrase with those obtained after exposure to the experimental environments, as shown in Table I, indicates that the potential enzymic activity is not markedly altered under these conditions. Values for all of the subjects were slightly higher, however, when measured during the control period than during exposure to the high pressure environment.

TABLE I. CARBONIC ANHYDRASE ACTIVITY OF DILUTED WHOLE BLOOD[1]

Subject	Control	Experimental[2]
M	.05446 ± .00326[3]	.04973 ± .00666
Bu	.05454 ± .00458	.04991 ± .01023
Ba	.05152 ± .00471	.04470 ± .00923

1. .01 ml of 1:25 dilution of blood per 1.5 ml reaction.
2. Second day of exposure to pressure.
3. m moles CO_2 hydrated per ml reaction mixture per min ± S D.

A t- test of the means of the sets of data indicates a reduction of total enzymic activity of possible significance under the high pressure environment. While six or more measurements were made on each of the samples, the limited data do not allow a clear cut decision concerning differences resulting from the experimental procedure. None of the experimental values is significantly different from the control for an individual subject.

The spectrum of isozymes of carbonic anhydrase in the blood of the subjects is depicted in Figure 1. Since the isozymes of human carbonic anhydrase have widely differing specific activities, changes in their ratios should give an indication of adaptive mechanisms put into operation. Under the condition studied, no qualitative or apparent quantitative alteration in the enzyme pattern was detected.

It is well established that a very large physiological excess of potential carbonic anhydrase activity exists in the blood (5) and in many other tissues (6). In view of such an excess the present study does not indicate that exposure to the experimental environ-

*Genesis I experiment, August-September 1963, Submarine Medical Research Laboratory.

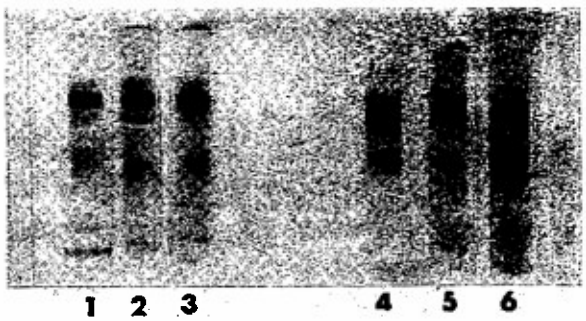

Figure 1. Disc Electrophoresis Columns Stained with Aniline Blue-Black as Discussed in Text at Times Indicated in Table I. Columns 1-3 control; 4-6 experimental. Order: left to right in each set—Bu, Ba, M.

ment significantly changes the total carbonic anhydrase activity with respect to the needs of the organism. On the other hand, it should be pointed out that measurements of potential activity do not define the actual amount of available enzyme called upon to operate under a particular physiological circumstance. The regulation of normal enzymic metabolism and alterations in control mechanisms as influenced by altered environments deserve further consideration.

SUMMARY

The total enzyme activity and distribution of isozymes do not indicate a change in the erythrocyte carbonic anhydrase system under seven atmospheres of pressure in a helium-oxygen environment that would seem to be of physiological significance. A possible small reduction in measurable activity is apparently far outweighed by the physiological excess of the available enzyme.

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13. ABSTRACT

The carbon dioxide hydrating ability of erythrocytes measured as carbonic anhydrase activity and the qualitative pattern of the isozymes of this enzyme group were studied in men exposed to a helium-oxygen environment at seven atmospheres pressure. No alteration in the enzyme or its activity of apparent physiological significance was detected.

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Enzyme activity						
Carbonic anhydrase after exposure to HeO_2						